DESIGN, DEVELOPMENT AND EVALUATION OF PULSATILE DRUG DELIVERY SYSTEM OF RAMIPRIL

A Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY
CHENNAI-600 032.

In partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY
PHARMACEUTICS

Submitted by

Register no: 261211251

Under the guidance of

Prof.K.Elango, M.Pharm., (Ph.D.)
Professor & Head
Department of Pharmaceutics

COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI- 600 003.

APRIL 2014

DEPARTMENT OF PHARMACEUTICS
COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI-600 003
TAMILNADU

DATE:

This is to certify that the dissertation entitled **"DESIGN, DEVELOPMENT AND EVALUTION OF PULSATILE DRUG DELIVERY SYSTEM OF RAMIPRIL"** submitted by the candidate with **Register No. 261211251** for a The Tamil Nadu Dr. M.G.R. Medical University examination is evaluated.

Evaluated

DEPARTMENT OF PHARMACEUTICS
COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI-600 003
TAMILNADU

CERTIFICATE

This is to certify that the dissertation entitled "**DESIGN, DEVELOPMENT AND EVALUATION OF PULSATILE DRUG DELIVERY SYSTEM OF RAMIPRIL"** submitted by the candidate with Reg No.261211251 in partial fulfillment of the requirements for the award of the degree of **MASTER OF PHARMACY** in **PHARMACEUTICS** by The Tamil Nadu Dr.M.G.R. Medical University is a bonafide work done by her during the academic year 2013-2014 .

Place: Chennai -03.

Date:

(Dr.A.Jerad Suresh)

DEPARTMENT OF PHARMACEUTICS
COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI-600 003
TAMILNADU

CERTIFICATE

This is to certify that the dissertation entitled **"DESIGN, DEVELOPMENT AND EVALUATION OF PULSATILE DRUG DELIVERY SYSTEM OF RAMIPRIL"** submitted by the candidate with Reg. No.261211251 in partial fulfillment of the requirements for the award of the degree of **MASTER OF PHARMACY** in **PHARMACEUTICS** by The Tamil Nadu Dr.M.G.R.Medical University is a bonafide work done by him during the academic year 2013-2014 under my guidance.

Place: Chennai -03.

Date:

Prof. K.Elango

ACKNOWLEDGEMENT

"Gratitude makes sense of our past, brings peace today and creates a vision for tomorrow"

I consider this as an opportunity to express my gratitude to all the dignitaries who have been involved directly or indirectly with the successful completion of this dissertation.

First of all we thank the **Almighty** for giving us strength, endurance and showering his blessing to undertake this project and pursue with full dedication and giving us courage always to do hard work.

We acknowledge our sincere thanks to **Prof. Dr.A. Jerad Suresh M.pharm.,Ph.D. .,MBA,** Principal, college of pharmacy ,Madras Medical College, Chennai, for his continuous support in carrying out our project work in this institution.

We consider ourselves very much lucky with profound privilege and great pleasure in expressing our deep sense of gratitude to work under the guidance of **Prof. Mr.K.Elango,M.pharm.,(Ph.D.),** Head, Department of pharmaceutics, College of Pharmacy, Madras Medical College, Chennai for his continuous guidance, supportive suggestion, innovative ideas, with constant inspiration, help and encouragement have always propelled us to perform better.

It is our privilege and honour to extend our gratitude and express our indebtedness to for his enduring support. He has been generous with providing facilities to carry out this work.

We express our sincere thanks and respectful regards to**,** in acknowledging all the facilities provided to us to carry out this work with great ease and precision.

We Express our deepest and very special thanks to**,** for allowing us to carry out this project successfully.

It ´s a great pleasure for us to acknowledge our sincere thanks to all my teaching staff members

Dr.N.Deattu,M.Pharm., Mrs.S.Daisychellakumari M.pharm., Mrs.R.DeviDamayanthi, M.Pharm., of the Department of Pharmaceutics, College of Pharmacy, Madras Medical College,Chennai-03.

We extend our thanks to all non-teaching staff members Mr.E.Arivazhagan, Mr.R.Marthandam, Mrs. R .Shankari Department of Pharmaceutics, College of Pharmacy, Madras Medical College,Chennai-03.

We would like to thank our mentors Muthulakshmi, Velmurugan, Venkatesh babu, Kishore kumar, Suhasini, Keerthana, Ramu, Chinna raja, David, Rajakumari, Catherin, Priya who stood beside us throughout our project.

We extend my cordial thanks to **our seniors** and to **our juniors** for their kind support and co-operation.

Most of all we would like to thank our **beloved parents** for their priceless support, love and encouragement throughout the entire tenure of this course.

LIST OF ABBREVIATIONS USED

API	: Active Pharmaceutical Ingredient
ACE	: Angiotensin Converting Enzyme
ADH	: Anti Diuretic Hormone
AT_1	: Angiotensin I Receptor
AT_2	: Angiotensin II Receptor
ARB	: Angiotensin Receptor Blocker
BCS	: Biopharmaceutical Classification System
BP	: British pharmacopoeia
β	: Beta
C	: Celsius
Conc	: Concentration
Cm	: Centimeter
et al	: and others
Fig	: Figure
FTIR	: Fourier Transform Infra Red
g	: Gram
hr	: Hours
HCl	: Hydrochloric acid
HPMC	: Hydroxy Propyl Methyl Cellulose
ie	: that is
IP	: Indian Pharmacopoeia
IPA	: Isopropyl Alcohol
IR	: Immediate Release
ICH	: International Conference on Harmonisation
M	: Molar
MCC	: Micro Crystalline Cellulose
mg	: Milligram
ml	: Millilitre
min	: Minute
µg	: Microgram
NaOH	: Sodium Hydroxide

nm	: Nanometre
NLT	: Not Less Than
NMT	: Not More Than
NC	: No Change
pH	: Negative logarithm of hydrogen ion concentration
PhEur	: European Pharmacopoeia
PVP	: Poly Vinyl Pyrrolidone
RAAS	: Renin Angiotensin Aldosterone System
rpm	: revolutions per minute
RH	: Relative Humidity
s	: seconds
SSG	: Sodium Starch Glycolate
SR	: Sustained Release
$t_{1/2}$	: Half Life
USP	: United States Pharmacopoeia
UV	: Ultra Violet
v	: volume
w	: weight
SEM	: Scanning Electron Microscopy

INDEX

DEDICATED TO MY FAMILY AND PROFESSION

INTRODUCTION

INTRODUCTION [1-8]

Oral drug delivery has been known for decades as the most widely used route of administration among all the routes. The reasons that the oral route achieved such popularity may be in part attributed to its ease of administration as well as the traditional belief. The oral controlled-release system shows a typical pattern of drug release in which the drug concentration is maintained in the therapeutic window for a prolonged period of time, thereby ensuring sustained therapeutic action shown in figure 1. There are certain conditions for which such a release pattern is not suitable that demand release of drug after a lag time. In other words, they require pulsatile drug delivery system (PDDS).

Pulsatile systems are designed in a manner that the drug is available at the site of action at the right time in the right amount. Pulsatile drug delivery systems (PDDS) have attracted attraction because of their multiple benefits over conventional dosage forms. Pulsatile drug delivery system is defined as the rapid and transient release of certain amount of molecules within a short time period immediately after predetermined off-release periods i.e. lag time[4]. These systems are designed according to the circadian rhythm or biological clock of the body.[3]

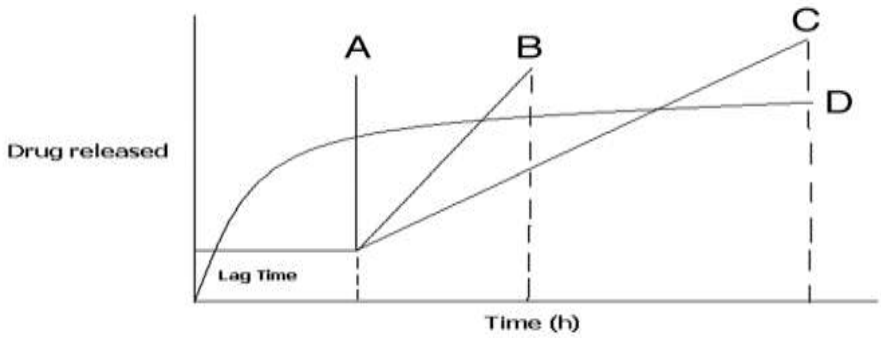


Figure 1.Schematic Representation of Different Drug Delivery SystemsWhere (A) sigmoidalrelease after lag time (B) delayed release after lag time (C) sustained release after lag time (D) extended release without lag time.[2]

Chronobiology [3]

Choronobiology is the science concerned with the biological mechanism of the disease according to a time structure. "chrono" pertains to time and "biology" pertains to the study , or science, of life.

Biological rhythms

Ultradian Rhythms

Oscillationsof shorter duration are termed Ultradian Rhythms (more than one cysle per 24 hrs). E.g. 90 minutes sleep cycle.

Infradian Rhythms

Oscoillations that are longer than 24hrs are termed as Infradian Rhythms (less than one cycle per 24hrs). E.g. Monthly Menstruation.

Circadian Rhythms

The term "circardian", coined by Franz halberg, comes from the Latin circa, "around", and diem of dies, "day", meaning literally "approximately one day". Our body appears to be genetically programmed to function on roughly a 24-hour cycle

Cycle of Circardian Rhythms

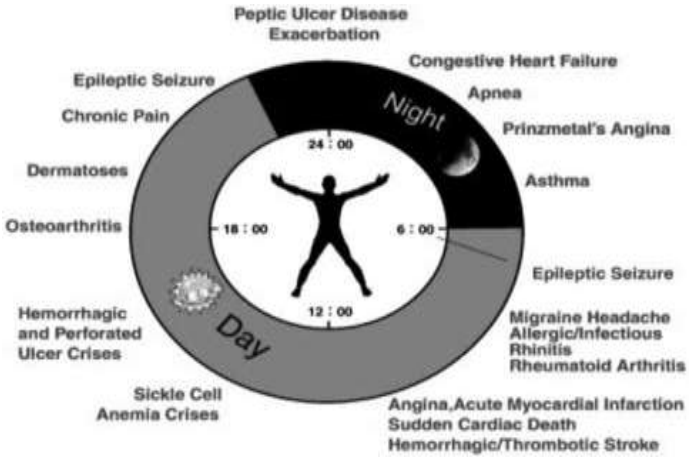


Figure 2. The Circadian Pattern of Diseases[4]

Chronopharmacokinetics

Chronopharmacokinetics involves study of temporal changes in drug absorption, distribution, metabolism and excretion. Pharmacokinetic parameters, which are conventionally considered to be constant in time, are influenced by different physiological functions displaying circadian rhythm. Circadian changes in gastric acid secretion, gastrointestinal motility, gastrointestinal blood flow, drug protein binding, liver enzyme activity, renal blood flow and urinary pH can play role in time dependent variation of drug plasma concentrations.

Chronotherapeutics

Chronotherapeutic is the discipline concerned with the delivery of drugs according to inherent activities of a disease over a certain period of time. It is becoming increasingly more evident that the specific time that patients take their medication may be even more significant than was recognized in the past.

Benefits of these Technologies[5]

- Once daily dose resembling multiple daily doses by releasing drugs in discrete bursts.
- Constant drug levels at the site of action and prevent the peak-valley fluctuations.
- Chance of development of drug resistance and tolerance can be reduced.
- Rate of release independent of pH, food and minimal potential for dose dumping.
- Facility to produce combination dosage forms, ease of combining pellets with different compositions or release pattern.
- Protection of mucosa from irritating drugs.
- Delivery profile designed to compliment circadian patter..
- Drug loss by extensive first pass metabolism is prevented.
- Reduced dose frequency, dose size and cost, which ultimately reduces side effects and local irritation, thereby improving patient compliance..

Limitations

- Multiple manufacturing steps.

- Homogenicity of the coated barrier is mandatory to assure the predictability of the lag time.
- Rupture time cannot be always adequately manipulated as it depends on the physicochemical properties of the polymer.
- Pulsatile delivery drugs are costly, raw material is not easily available.
- Dosage form design requires highly educated professionals.
- Technologies employed and the equipment used is complicated.

Diseases presently targeted for chronopharmaceutical formulations are those for which there are enough scientific backgrounds to justify PDDS- compared to the conventional drug administration approach . They include: hypercholesterolemia, asthma, cancer, duodenal ulcer, arthritis, diabetes, neurological disorders, cardiovascular diseases (e.g., hypertension and acute myocardial infarction) and colonic delivery[6].

On which cases or circumstance pulsatile drug delivery is used they are listed below[7].

1) Chronopharmacotherapy of diseases whichshows circadian rhythms in their pathophysiology.

2) Avoiding the first pass metabolism, e.g. protein and peptides

3) For which the tolerance is rapidly exists,

4) For targeting specific site in intestine, e.g. colon,

5) For time programmed administration of hormone and drugs,

6) For drugs having the short half life

Diseases Requiring Pulsatile Drug Delivery [8]

1. Peptic ulcer: Acid secretion is high in the afternoon and at night.

2. Asthma: Precipitation of attacks during night or at early morning hour

3. Cardiovascular diseases: BP is at its lowest during the sleep cycle and rises steeply during the early morning period.

4. Arthritis: Pain in the morning and more pain at night

5. Diabetes mellitus: Increase in the blood sugar level after meal

6. Attention deficit syndrome: Increase in DOPA level in afternoon

Methodologies for Pulsatile Drug Delivery Systems[4]

1. Time Controlled Pulsatile Release

A. Single Unit System

- Capsular systems
- Port systems
- Delivery by series of stops
- Delivery by solubility modulation
- Delivery by reservoir systems with erodible or soluble barrier coatings

B. Multiparticulate Systems

- Pulsatile system based on rupturable coating
- Time controlled explusion system
- Pulsatile delivery by change in membrane permeability

2. Stimuli Induced

A. Thermo Responsive Pulsatile Release

B. Chemical Stimuli Induced Pulsatile Release

- Glucose responsive insulin release device
- Inflammation induced pulsatile release
- Drug release from intelligent gel responding to antibody concentration
- pH sensitive drug delivery system

C. External Stimuli Pulsatile Release

- Electro responsive pulsatile release
- Micro electro mechanical systems
- Magnectically induced pulsatile release

3. Pulsatile Release Systems for Vaccine and Hormone Product

Classification of Pulsatile Drug Delivery Technologies Based on Route of Administration[6]

1. **By Transdermal Route**

- Crystal reservoior technology

2. **By Oral Route**

- Pulsincap
- Diffucaps
- Egalet
- Orbexa
- Minitabs
- Contin
- SODAS
- IODAS
- IPDAS
- Geomatrix
- Pulsys

TIME CONTROLLED PULSATILE RELEASE

A. Single Unit System

Capsular system

A general system consists of an insoluble capsule body housing a drug ,soluble cap and a plug. The plug is removed after a predetermined lag time owing to swelling, erosion, or dissolution. Pulsincap was developed by R.R. Scherer International Corporation, Michigan, US[3] is an example of such a system that is made up of a water-insoluble capsule body filled with drug formulation (Fig. 2). The body is closed at the open end with a swellable hydrogel plug. Upon contact with dissolution medium or gastro-intestinal fluids, the plug swells, pushing itself out of the capsule after a lag time. This is followed by a rapid drug release. The lag time can be controlled by manipulating the dimension and the position of the plug.

For water-insoluble drugs, a rapid release can be ensured by inclusion of effervescent agentsordisintegrants. The plug material consists of insoluble but permeable and swellable polymers (e.g. polymethacrylates), erodible compressed polymers (e.g. hydroxypropylmethyl cellulose, polyvinyl alcohol, polyethylene oxide), congealed melted polymers (e.g. Saturated polyglycolated glycerides, glycerylmonooleate), and enzymatically controlled erodible polymer (e.g., pectin). These formulations were well tolerated in animals and healthy volunteers, and there were no reports of gastro-intestinal irritation. However, there was a potential problem of variable gastric residence time, which was overcome by enteric coating the system to allow its dissolution only in the higher pH region of small intestine .

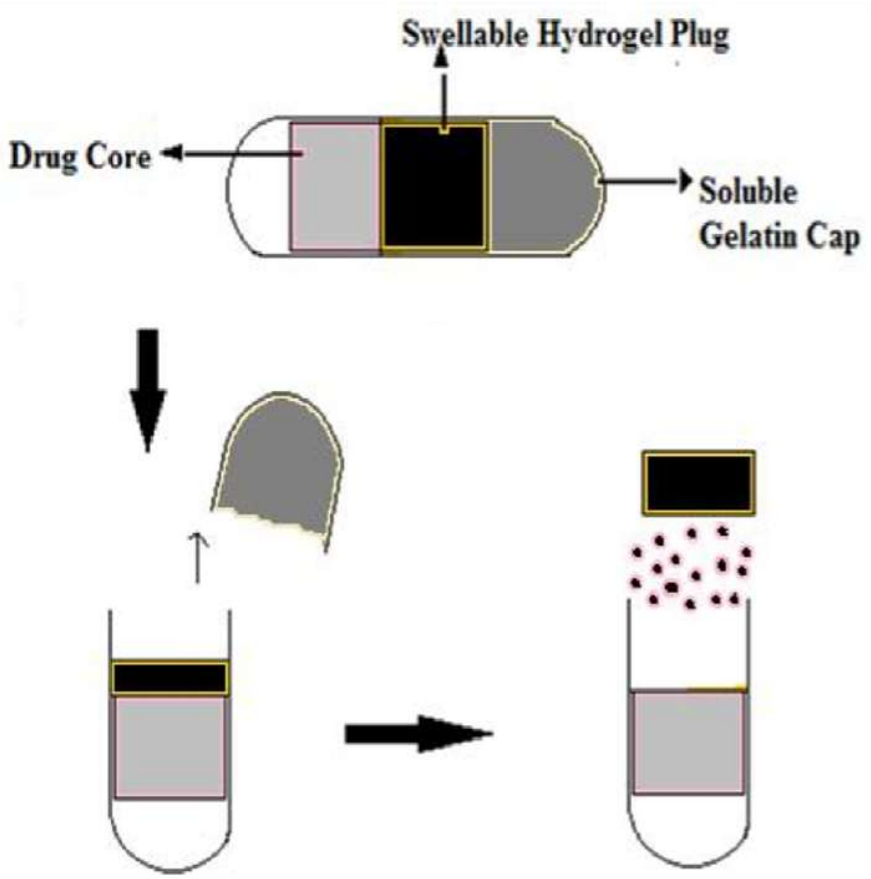


Figure 3.Pulsincap

Port System

The Port system was developed by Therapeutic system research laboratory Ann Arbor, Michigan, USA, This system consists of a gelatin capsule coated with a semi permeable membrane (cellulose acetate). Inside the capsule is an insoluble plug, an osmotically active agent along with the drug formulation. (24). When this cap comes into contact with GI fluids, water diffuses across the semi permeable membrane, resulting in increased pressure inside that ejects the plug after a predetermined lag time. The lag time is controlled by the thickness of the coating. The system was proposed to deliver methylphenidate for the treatment of attention deficit hyperactivity disorder (ADHD) in school-age children. Thissystem avoided second time dosing, which was beneficial for school children during daytime.

Delivery by Series of Stops

This system is described for implantable capsules. The capsule contains a drug and a water-absorptive osmotic engine that are placed in compartments separated by a movable partition. The pulsatile delivery is achieved by a series of stops along the inner wall of the capsule. These stops obstruct the movement of the partition but are overcome in succession as the osmotic pressure rises above a threshold level. The number of stops and the longitudinal placements of the stops along the length of the capsule dictate the number and frequency of the pulses, and the configuration of the partition controls the pulse intensity.

Delivery by Solubility Modulation

Solubility modulator of system provides pulsed delivery of variety of drugs. The system was especially developed for delivery of salbutamol sulphate that contained sodium chloride as modulating agent. Amount of sodium chloride was less than the amount needed to maintain saturation in a fluid enters the osmotic device. The pulsed delivery is based on drug solubility. Solubility has solubility of 275mg/ml in water and 16mg/ml in saturated solution of sodium chloride, while sodium chloride has solubility of 321 mg/ml in water, and its saturation solubility is 320mg/ml. these

values show that the solubility of the drug is function of the modulator concentration, while the modulators solubility is largely independent of drug concentration. The modulating agent can be a solid organic acid, inorganic salt, or organic salt. Ratio of drug/modulator may be varied to control zero order release period and commence pulsed release. After the period of zero-order release, the drug is delivered as one large pulse.

Delivery by Reservoir Systems with Erodible (or) Soluble Barrier Coatings

Most of the pulsatile delivery systems are reservoir devices coated with a barrier layer. This barrier erodes or dissolves after a specific lag period, and the drug is subsequently released rapidly from reservoir core. The lag time depends on the thickness of the coating layer.

B. Multiparticulate System

Pulsatile System Based on Rupturable Coating

These systems depend on disintegration of the coat for the release of drug.The pressure needed for the rupture of the coating is achieved by effervescent excipients, swelling agents, osmotic pressure.

Time Controlled Explosion System

Multiparticulate system where drug is coated on non-pareil sugar seeds followed by a swellable layer and an insoluble top layer coating (Ueda et al., 1994). The swelling agents used include superdisintegrants like sodium carboxymethyl cellulose, sodium starch glycolateetc, and polymers like polyvinyl acetate, polyacrylic acid etc. Alternatively, effervescent system comprising a mixture of tartaric acid, citric acid and sodium bicarbonate may also be used. Upon ingress of water, the swellable layer expands, resulting in rupture of film with subsequent rapid drug release.

Pulsatile Delivery by Change in Membrane Permeability

The permeability and water uptake of acrylic polymers with quaternary ammonium groups can be influenced by the presence of different counter-ions in the medium. Several delivery systems based on this ion exchange have been developed.

STIMULI INDUCED

In these systems there is release of the drug after stimulation by any biological factor like temperature, or any other chemical stimuli.

A. Thermo-Responsive Pulsatile Release

Thermo-responsive hydro gel systems have been developed for pulsatile release. In these systems the polymer undergoes swelling or deswelling phase in response to the temperature which modulate drug release in swollen state.

B. Chemical Stimuli Induced Pulsatile Systems

Glucose Responsive Insulin Release Device

Several systems have been developed which are able to respond to changes in glucose concentration. One such system includes pH sensitive hydrogel containing glucose oxidase immobilized in the hydrogel. When glucose concentration in the blood increase glucose oxidase converts glucose into gluconic acid which changes the pH of the system. This pH change induces swelling of the polymer which results in insulin release. Insulin by virtue of its action reduces blood glucose level and consequently gluconic acid level also gets decreased and system turns to the deswelling mode thereby decreasing the insulin release.

Inflammation Induced Pulsatile Release Device

Yui and co-workers focused on the inflammatory-induced hydroxyl radicals and designed drug delivery systems, which responded to the hydroxyl radicals and degraded in a limited manner.

Drugs Release from Intelligent Gels Responding to Antibody Concentration

Special attention was given to antigen-antibody complex formation as the cross-linking units on the gel, since such interactions are very specific. Utilizing the difference in association constants between polymerized antibody and naturally derived antibodies towards specific antigens, reversible gel swelling/deswelling and drug permeation changes occurs.

pH sensitive drug delivery system

This type of PDDS contains two components. The first is fast release type while the other is pulsed release which releases the drug in response to change in pH. In case of pH dependent system, advantage has been taken of the fact that there exists different pH environment at different part s of the gastrointestinal tract. By selecting the pH dependent polymers drug release at specific location can be obtained. Examples of pH dependent polymers include cellulose acetate phthalate, polyacrylates, and sodium carboxymethylcellulose. These polymers are used as enteric coating materials so as to provide release of drug in the small intestine.

III. External Stimuli Pulsatile Release:

This system was divided into three subparts and is discussed below.

1. Electro responsive pulsatile release

Electrically responsive delivery systems are prepared from polyelectrolytes (polymers which contain relatively high concentration of ionisable groups along the backbone chain) and are thus, pH-responsive as well as electro-responsive. Examples of naturally occurring polymers include hyaluronic acid, chondroitin sulphate, agarose, carbomer, xanthan gum and calcium alginate. The synthetic polymers are generally acrylate and meth acrylate derivatives such as partially hydrolyzed polyacrylamide, polydimethylaminopropyl acrylamide.

2. Micro electro mechanical systems (MEMS)

A micro fabricated device has the ability to store and release multiple chemical substances on demand by a mechanism devoid of moving its parts. The digital capabilities of MEMS may allow greater temporal control over drug release compared to traditional polymer-based systems. Another development in MEMS technology is the microchip. The microchip consists of an array of reservoirs that extend through an electrolyte-impermeable substrate. The prototype microchip is made of silicon and contains a number of drug reservoirs, each reservoir is sealed at one end by a thin gold membrane of material that serves as an anode in an electrochemical reaction and dissolves when an electric potential is applied to it in an electrolyte solution. The reservoirs are filled with any combination of drug or drug mixtures in any form (i.e. solid, liquid or gel). When release is desired, an electric potential is applied between an anode membrane and a cathode, the gold membrane anode dissolves within 10- 20 seconds and allows the drug in the reservoir to be released. This electric potential causes oxidation of the anode material to form a soluble complex with the electrolytes which then dissolves allowing re lease of the drug. Complex release patterns (such as simultaneous constant and pulsatile release) can be achieved from the microchips. Microchip has the ability to control both release time and release rate.

3. Magnetically induced pulsatile release

The use of an oscillating magnetic field to modulate the rates of drug release from polymer matrix was one of the old methodologies. Magnetic carriers receive their magnetic response to a magnetic field from incorporated materials such as Magnetite, Iron, Nickel, Cobalt etc. For biomedical applications, magnetic carriers must be water-based,biocompatible, non-toxic and non-immunogenic mechanistic approach based on magnetic attraction is the slowing down of oral drugs in the gastrointestinal system. This is possible by filling an additional magnetic component into capsules or tablets. The speed of travel through the stomach and intestines can then be slowed down at specific positions by an external magnet, thus changing the timing and/ or extent of drug absorption into stomach or intestines.

IV. Pulsatile Release Systems For Vaccine And Hormone Products

Vaccines are traditionally administered as an initial shot of an antigen followed by repeated booster shots to produce protective immunity. The frequency of the booster shots, and hence the exact immunisation- schedule is antigen dependent. Also, co-administration of vaccine adjuvant is often required to enhance the immune response to achieve protective immunity. PDDS offer the possibility of single-shot vaccines if initial booster release of the antigen can be achieved from one system in which timing of booster release is controlled. Vizcarra et al. found in nutritionally anoestrous cows, GnRadministered in pulses of 2 mg over 5 min every hour for 13 days produced a higher frequency of luteal activity by 13th day than cows given continuous infusions or pulses every 4 Hr.

RAMIPRIL[11]

Ramipril is a potent ACE inhibitor used in the treatment of hypertensive disease. It is a highly lipophilic (log p octanol/water, 3.32) and poorly water soluble drug, with absolute bioavailability of 28-35% and half-life 2-4 hours[4]. It undergoes significant 'first pass' metabolism. Ramipril is a prodrug and is converted into an active metabolite ramiprilat by liver esterase enzymes. Ramiprilat is mostly excreted by the kidneys. The half-life of ramiprilate is variable, and is prolonged by heart and liver failure, as well as kidney failure.

Ramipril is marketed in India under the brand names of Cardace, Zigpril and Zorem. Single doses of Ramipril of 2.5 - 20 mg produce approximately 60 - 80 % inhibition of ACE activity 4hours after dosing with approximately 40 - 60% inhibition after 24 hours.

MICROSPHERES[9-11]

Oral drug delivery has been known for decades as the most widely used route of administration among all the routes. The reasons that the oral route achieved such popularity may be in part attributed to its ease of administration as well as the traditional belief[1].

Drug delivery systems (DDS) that can precisely control the release rates or target drugs to a specific body site have had an enormous impact on the health care system. The ideal drug delivery system delivers drug at rate decided by the need of the body throughout the period of treatment and it provides the active entity solely to the site of action. So, carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as microspheres, nanoparticles, liposomes, etcwhich modulates the release and absorption characteristics of the drug.

Types of drug delivery system are,

- LIPOSOME
- NIOSOME
- NANOPARTICAL
- **MICROSPHERE**

MICROSPHERES

There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carries for drugs. Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 μm[9]

Microspheres used usually are polymers. They are classified into two type[9]

1. **Synthetic Polymers**

a. Non-biodegradable polymers

e.g. Polymethacrylayte, Acrolein, glycidylmethacrylate,epoxypolymers

b. Biodegradable polymers

e.g. Lactides, Glycolides and their co polymers poly alkyl cyano acrylates, poly anhydride

2. Natural Polymers

a. Proteins : Albumin , Gelatin And Collagen

b. Carbohydrates : Agarose, Carrageenan , Chitosan, Starch

c. Chemical Modified Carbohydrates:Polydextran,Polystarch.

ADVANTAGES

1. Microspheres provide constant and prolonged therapeutic effect.
2. Reduces the dosing frequency and thereby improve the patient compliance.
3. They could be injected into the body due to the spherical shape and smaller size.
4. Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects.
5. Microsphere morphology allows a controllable variability in degradation and drug release.

LIMITATION

1. The modified release from the formulations.
2. The release rate of the controlled release dosage form may vary from a variety of factors like food and the rate of transit though gut.
3. Differences in the release rate from one dose to another.
4. Controlled release formulations generally contain a higher drug load and thus any loss of integrity of the release characteristics of the dosage form may lead to potential toxicity.
5. Dosage forms of this kind should not be crushed or chewed.

TYPES OF MICROSPHERES

- Bioadhesive microspheres
- Magnetic microspheres
- Floating microspheres
- Radioactive microspheres
- Mucoadhesive microspheres

- Polymeric microspheres

BioadhesiveMicrospheres

The term “bioadhesion” describes materials that bind to biological substrates, such as mucosal members. Adhesion of Bioadhesive drug delivery devices to the mucosal tissue offers the possibility of creating an intimate and prolonged contact at the site of administration. This prolonged residence time can result in enhanced absorption and in combination with a controlled release of drug also improved patient compliance by reducing the frequency of administration.

Magnetic Microspheres

This kind of delivery system is very much important which localizes the drug to the disease site. In this large amount of freely circulating drug can be replaced by smaller amount of magenetically targeted drug. Magnetic carriers receive magnetic responses to a magnetic field from incorporated materials that are used for magnetic microspheres are chitosan, dextran etc. The different type are Therapeutic magnetic microspheres are used to deliver chemotherapeutic agent to liver tumour. Drugs like protein and peptides can also be targeted through this system.

Floating Microspheres

In floating types the bulk density is less than the gastric fluid and so remains buoyant in stomach without affecting gastric emptying rate. The drug is released slowly at the desired rate, if the system is floating on gastric content, increases gastric residence and fluctuation in plasma concentration. It also reduces chances of striking and dose dumping and produces prolonged therapeutic effect.

Radioactive Microspheres

Radioactive microspheres deliver high radiation dose to the targeted areas without damaging the normal surrounding tissues. It differs from drug delivery, as radio activity is not released from microspheres but acts from within a radioisotope typical

distance and the different kinds of radioactive microspheres are α emitters, β emitters, γ emitters.

Mucoadhesive Microspheres

Mucoadhesive microspheres which are of 1-1000nm in diameter and consisting either entirely of a mucoadhesive polymer or having an outer coating of it and coupling of mucoadhesive properties to microspheres properties to microsphere has additional advantages, e.g. Efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer, specific targeting of dug to the absorption site achieved by anchoring plant lectins, bacterial adhesion and antibodies, etc. on the surface of the microspheres.

Polymeric Microspheres

The different types of polymeric microspheres can be classified as:

- Biodegradable polymeric microspheres
- Synthetic polymeric microspheres

Biodegradable Polymeric Microspheres

Biodegradable polymers prolongs the residence time when contact with mucosmembrane to its high degree of swelling property with aqueous medium, results gel formation. The rate and extent of drug release is controlled by concentration of polymer and the release pattern in a sustained manner.

Synthetic Polymeric Microspheres

The interest of synthetic polymeric microspheres are widely used in clinical application, moreover that also used as bulking agent, fillers, embolic particle, drug delivery vechilesetc and proved to be safe and biocompatible. But the main disadvantage of these kinds of microspheres, are tend to migrate away from injection site and lead to potential risk, embolism and further organ damage.

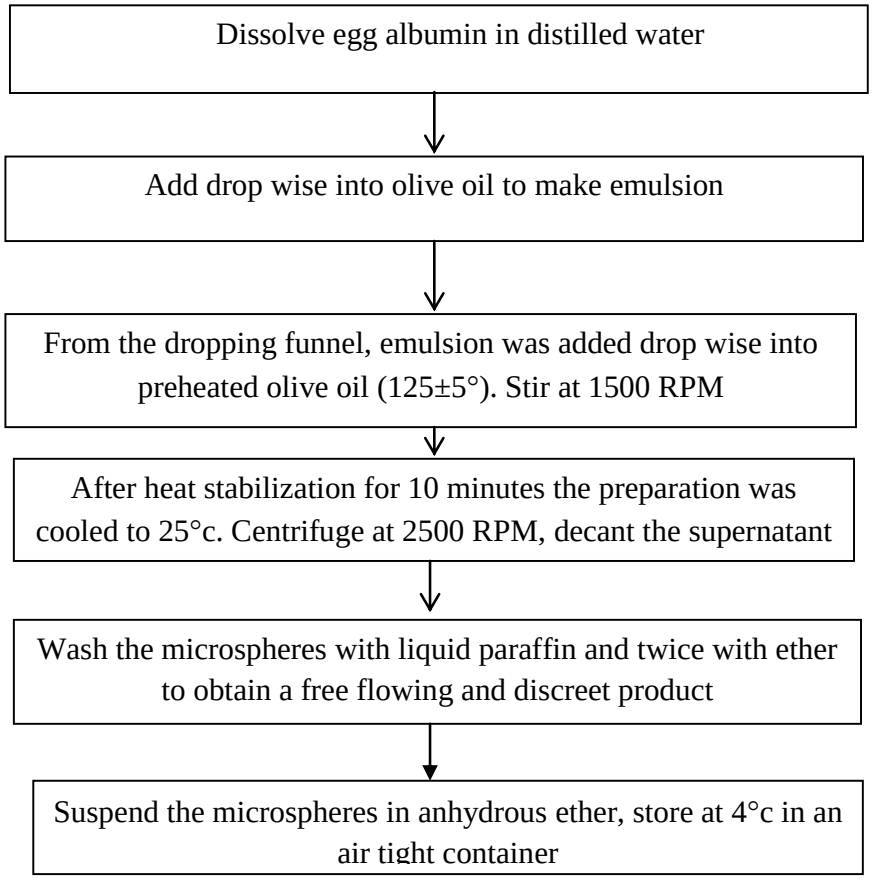
ALBUMIN[12, 13]

Albumin – natural protein is emerging as a versatile protein carrier for drug targeting and for improving the pharmacokinetic profile of peptide or protein-based drugs. Albumin is an acidic, very soluble protein that is extremely robust, it is stable in the pH range of 4-9, soluble in 40% ethanol, and can be heated at 60^0c for up to 10h without deleterious effects. Albumin microspheres are generally prepared by chemical cross linking or by addition of an organic solvent and stabilization at elevated temperatures.

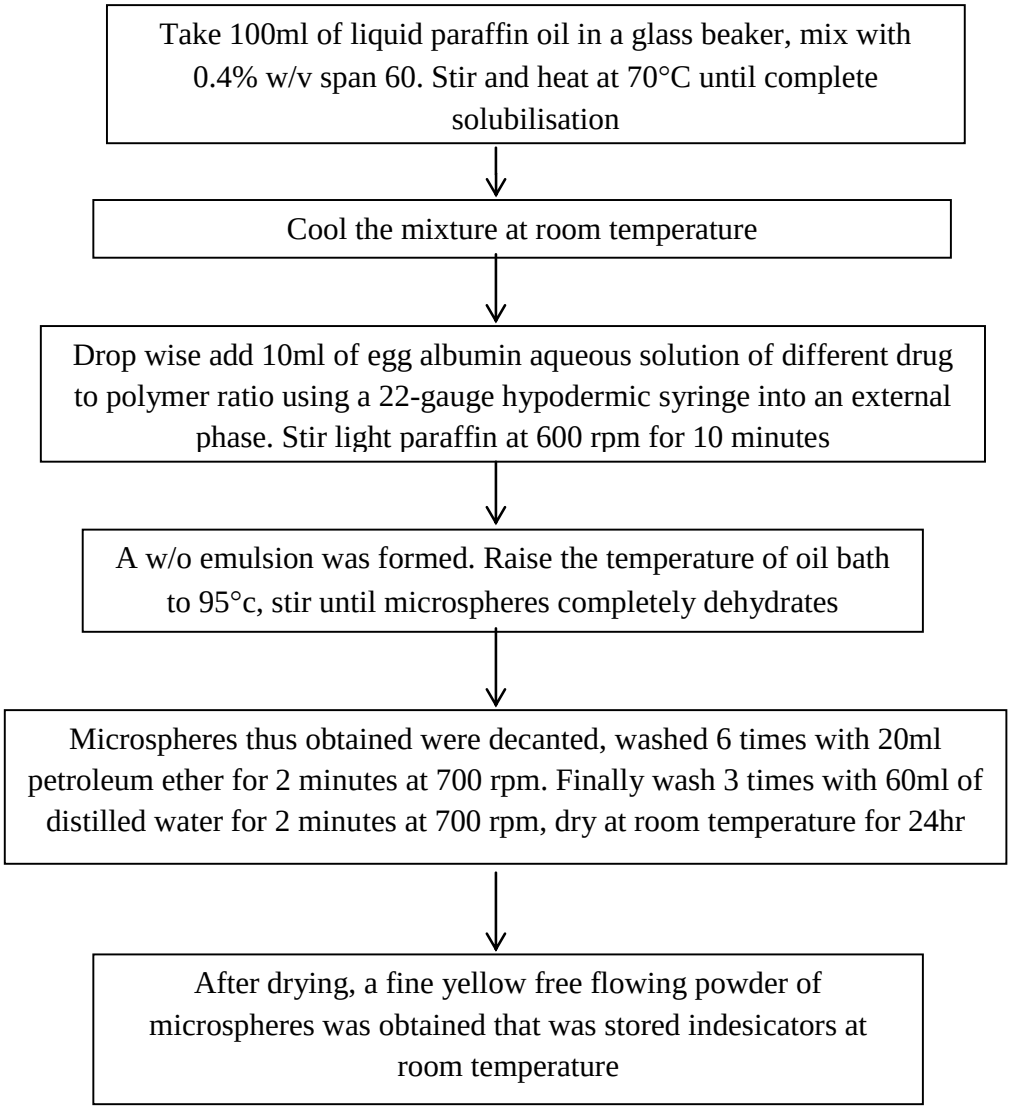
Preparation of albumin microspheres can be done by suitable methods like:

- Protein gelation technique
- Single emulsion polymerization technique
- Double emulsion polymerization technique
- Multiple emulsion polymerization technique
- Solvent evaporation technique
- Sonication technique
- Spray and freeze drying technique
- Emulsification – heat stabilization technique

Protein Gelation Technique



- **Single Emulsion Polymerization Technique**



Double Emulsion Polymerization Technique

A double emulsion is usually prepared in two main modes,

Mode 1: one step emulsification

Mode 2: two step emulsification

In one step emulsification mode a strong mechanical agitation is used for the water phase containing a hydrophilic surfactant and an oil phase containing large amounts of hydrophobic surfactant. Due to this a w/o emulsion is formed which quickly inverts to form a w/o/w double emulsion.

A two-step procedure is reported where the primary emulsion can be formed as a simple w/o emulsion which is prepared using water and oil solution with a low HLB (hydrophilic-lipophilic balance) surfactant. In the second step, the primary emulsion (w/o) is re-emulsified by aqueous solution with a high HLB surfactant to produce a w/o/w double emulsion.

Multiple Emulsion Polymerization Technique

Multiple emulsion method involved formation of (o/w) primary emulsion (non-aqueous drug solution in polymer solution) and then addition of primary emulsion to external oily phase to form o/w/o emulsion followed by either addition of crosslinking agent (glutataldehyde) and evaporation of organic solvent. This method of preparation is ideal for incorporating poorly aqueous soluble drug, thus enhancing its bioavailability.

Solvent Evaporation Technique

This process is carried out in a liquid manufacturing vehicle. The albumin microspheres are dispersed in a volatile solvent, which is immiscible with the liquid manufacturing with the liquid manufacturing vehicle phase. A core material to be microencapsulated is dissolved or dispersed in the coating polymer solution. With agitation the coating material mixture is dispersed in the liquid manufacturing vehicle phase to obtain the appropriate size microsphere. The mixture is then heated if

necessary to evaporate the solvent. The solvent evaporation technique to produce microsphere is applicable to wide variety of core material. The core material either water soluble or water insoluble materials. Solvent evaporation involves the formation of an emulsion between polymer solution and an immiscible continuous phase whether aqueous (o/w) or non-aqueous.

Sonication Technique

As the technique name itself is self-explanatory, it just involves a simple sonication for certain period of time till a desired size of albumin microspheres is obtained. The albumin solution of desired concentration is taken which is sonicated. To this add the drug which will then form intrachain cross-link with cysteine residues of albumin chains.

Spray Drying Technique

In spray drying the polymer is first dissolved in a suitable volatile organic solvent such as dicholoromethane,acetone, etc. the drug in the solid form from is then dispersed in the polymer solution under high-speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small dropletsor the fine mist from which the solvent evaporate instantaneously leading formation of the microspheres in a size range 1-100µm. microparticles are separated from the hot air by means of the cyclone separator while the trace of the solvent is removed by vaccum drying. One of the major advantages of process is fessibility of operation under aseptic conditions. This process is rapid and leads to the formation of porous micro particles.

LITERATURE REVIEW

LITERATURE REVIEW

Listair CR *et al*[14].developed a chronopharmaceutical capsule drug delivery system capable of releasing drug after predetermined time delays. The drug formulation is sealed inside the capsule body by an erodible tablet. The release time is determined by erodible tablet erosion rate and increases as the content of an insoluble excipient (dibasic calcium phosphate) and of gel forming excipient (HPMC) increases. Programmable pulsatile release has been achieved from a capsule over a 2-12 hrs period, consistent with the demands of chronotherapeutic drug delivery system.

Anil kumar *et al*[15].developed and evaluated an oral pulsatile drug delivery system to mimic the circadian rhythm of the disease by releasing the drug with a distinct predetermined lag time of 5hrs(± 0.25hrs). The pulsincap formulation of metoprolol provides time controlled release to treat the nocturnal symptoms of hypertension and angina pectoris. Metoprolol succinate granules were prepared by wet granulation technique using a gugar gum polymer in different ratios.

Viharmoturi *et al*[16].developed and evaluated the modified pulsincap of an cardiovascular drug, Ramipril. Ramipril granules with different ratios of polymer plugs such as HPC(hydroxyl propyl cellulose), HPMC(hydroxyl propyl methyl cellulose k-15), and chitosan. The granules were prepared by wet granulation technique and evaluated for angle of repose, cars index, in vitro release, content uniformity. The modified pulsincap which contain HPC, Chitosan as polymer plugs had showed the required lad time, provided the immediate release of Ramipril and frequency of dosing is reduced.

Hakan Eroglu *et al*[17].formulated dexamethasone sodium phosphate (DSP) bovine serum albumin microspheres .microspheres were prepared by emulsion polymerization technique. An aqueous solution of gularaldehyde (25%w/v) was used as the crosslinking agent in two different amounts. The release time DSP was found to be extended in the series containing 15% DSP with the increase in the amount of glutaraldehyde used. Also it was observed that the release time is extended in series prepared using 0.5 ml glutaraldehyde with the amount of DSP.

Meena.A *et al*[18]. investigated the development of pulsatile drug delivery system based on an insoluble capsule body filled with lornoxicam microcapsules and sealed HPMCK4M plug. Optimized microcapsule formulations were selected by percentage drug content, invitro studies. Plug of varying thickness and hardness were prepared by direct compression which was then placed in the capsule opening. Pulsincap F2 release was found to be diffusion controlled and followed zero order kinetics.

Sukanya.M *et al*[19].developed a pulsatile drug delivery system based on an insoluble capsule body filled with simvastatin microspheres and sealed with HPMCK4M plug. Microspheres were prepared by quasi emulsion solvent diffusion method of the spherical crystallization technique. Optimized microsphere formulation were selected by percentage drug content, *invitro* studies. The plug of varying thickness and hardness were prepared by direct compression which was then placed in the capsule opening. The drug release from all the pulsing caps followed zero order kinetics .

Senthilnathan. B *et al*[20]. formulated and evaluated the pulsincap for anti diabetic dug Glibenclamide to control the increased blood glucose level after food consumption in diabetic patient by allowing the drug to release immediately after a lag time (after meals). Microsponges of different concentrations were prepared selected the best formulation for the development of pulsincap and the optimized microsponges were subjected to scanning electron microscopy, FT-iR , and in vitro studies.

Najmuddin .M *et al* [21].developed and evaluated pulsatile drug delivery of flurbiprofen. The basic design consists of an insoluble hard gelatin capsule body, filled with eudragit microsphere of flubiprofen and sealed with a hydrogel plug. The entire device was enteric coated, so that the variability in gastric emptying time can be overcome and a colon-specific release can be achieved. Flurbiprofen microsphere was prepared by using ratio of Drug :Eudragit L-100 : Eudragit S-100 (1:1:2). Different hydrogel polymers were used as plugs (Guar gum, HPMC, Sodium alginate) to maintain a suitable lag period and it was found that the drug release was controlled by the proportion of polymers used. In vitro release studies of pulsatile device revealed that, increasing the hydrophilic polymer content resulted in delayed release of flurbiprofen from microsphere. Programmable pulsatile, colon-specific

release has been achieved from a capsule device over a 2-15h period, consistent with the demands of chronotherapeutic drug delivery.

Amol M *et al*[22].designed and evaluated the pulsatile drug delivery system of Atenolol for chronomodulated therapy. Pulsatile release tablet comprises of a drug containing core and pH sensitive polymeric coating capable of delaying drug release and providing gastric resistance to overcome gastric emptying variability, thus allowing colon delivery to be pursued according to the time- dependent approach. To evaluate different pH sensitive polymers (Eudragit S-100, ethyl cellulose, sodium alginate) at different ratio in developing a suitable dosage form, exhibiting a no drug release in upper region of gastrointestinal tract (GIT) in order to provide site specificity as well as time controlled formulation.

Sindhu Abraham et al[23].studied the time dependent formulation named 'Modified Pulsincap' that would ensure chronotherapeutic delivery of Diclofenac sodium (DFS) in the colon for the relief of rheumatoid arthritis.Bodies of gelatin capsules were made insoluble by formaldehyde treatment. Drug loaded pellets were prepared by Extrusion-Spheronization technique. The pellets equivalent to 100mg of the drug were filled into the treated capsule shells, plugged with hydrogel polymers Hydroxypropyl Methylcellulose, Hydroxypropyl cellulose and Sodium Alginate at different concentrations and completely enteric coated with 5% Cellulose Acetate Phthalate. The ability of Modified Pulsincap to provide colon specific drug delivery was assessed by In vitro drug release studies in simulated gastric fluid for 2 hrs, simulated intestinal fluid for 3 hrs& simulated colonic fluid for 7 hours. The formulation was found to be intact for 2 hours in simulated gastric fluid. The accelerated stability studies carried out for three months as per ICH guidelines proved that the formulations were stable and thus Diclofenac Sodium could be successfully colon targeted with a time dependent formulation such as 'Modified Pulsincap'.

Masareddy R et at[24].formulated and evaluated chronomodulatedpulsincap drug delivery system of Aceclofenac for arthritis. Aceclofenac microcapsules prepared by solvent evopration method using three different drug : polymer ratio (1:1, 1:2, 1:3) were subjected to various physic-chemical studies. The optimized formulation MC2

was filled into formaldehyde treated gelatin capsule using different concentrations (10%, 20% and 30%) of guar gum and methyl cellulose as hydrogel plugs. Further modified capsules were coated with Eudragit S-100 as enteric coating and HPMC as swellable coating by dip coat method. The prepared modified pulsincaps evaluated for physicochemical and release parameters.

Howard NE Stevens et al[25].evaluated the pulsincap to provide regional delivery to dofetilide to the human G tract. Dofetilife is a well absorbed drug, but showed a reduction in observed bioavailability when delivered from the pulsincap formulations, particularly at more distal GI tarct sites. Dispersion of the drug from the soluble excipient used and consistency of absorption from the colon was discussed. In these studies the effects of te degree of dispersion versus the site of dispersion could not be ascertained, nevertheless the scintigraphic analysis demonstrated good in vitro- in vivo correlation for time of release from pulisncap preaprations. The combination of scintigraphic and pharmacokinetic analysis permits identification of the site of drug release from the dosage form and pharmacokinectic parameters to be studied in man in a non-invasive manner.

Neha Manish Munot *et al*[26].studied Modified Pulsincaps and compression coated tablets of aceclofenac, an steroidal anti-inflammatory drug used for the treatment of rheumatoid arthritis were developed to target drug release in the colon. Pulsincaps were formulated by treating bodies of hard gelatin capsules with formaldehyde and caps were untreated. Aceclofenac was incorporated into these specialized capsule bodies which were plugged with hydrogels like guargum, acacia, gelatin and sodium alginate separately and in combination. Pulsincaps were evaluated for lag time, qualitative test for free formaldehyde and in *vitro* drug release studies. Compression coated tablets of aceclofenac were developed using Guar gum to deliver drug to colon due to its release retarding property and susceptibility to microbial degradation by coloniclike *Bacteroides species.* These tablets were evaluated for various parameters like hardness, friability, drug content, *in vitro* drug release studies in simulated colonic fluid containing Male Wistar Rats colonic contents. The findings of the present study conclusively state that developed dosage forms are promising for colon

targeting of aceclofenac to synchronize the chronobiological symptoms for effective treatment of rheumatoid arthritis.

Puttarajesh kumar *et al*[27].formulated and evaluated pulsatile drug delivery system to achieve time release of Verapamil HCl. The basic design consists of an insoluble hard gelatin capsule body filled with physical mixture of Verapamil HCl with HPMC and Guar gum, lactose as channeling agent and sealed with a Sodium alginate and xanthan gum plug. The Verapamil HClpulsincaps were prepared by physical mixture method with lactose by varying drug to polymer ratio and evaluated for the micromeretic property, percentage yield, drug content, IR and *in vitro* release study. A hydrogel polymer Sodium alginate and Xanthan gum was used as plugs to maintain a suitable lag period. The *in vitro* release study were carried out using pH 1.2 buffer for a period of 2 h then 7.4 pH phosphate buffer for a period of 10 h. Formulation GS3 showing 94.5% drug release at 12 h with 3h lag time was selected as an optimized formulation. The programmable pulsatile release has been achieved from prepared formulation over a 12 h period, consistent with the demands of Pulsincap drug delivery.

Srinivas L *et al*[28].studied Ibuprofen as pulsincap technique. The prepared capsules were evaluated for uniformity of weight, drug content and in vitro release. Promising results indicated the usefulness of the pulsincap technique for controlled release of ibuprofen.

LITERATURE REVIEW OF MICROSPHERES

Dinalpatel *et al*[29].formulated and evaluated the levosalbutamol sulphate loaded microspheres of chitosan and microspheres of Flax seed Mucilage in which both the polymers were checked as potential mucoadhesive agents and crosslinking was done with Glutaraldehyde , prepared by spray drying method, for prolonging drug release for asthma therapy.Microspheres having chitosan polymer : Drug ratio of 2:1 and 0.6% of Glutaraldehyde and that of Flax seed Mucilage : Drug ratio of 3:1 and 0.9% of glutaraldehyde had food mucoadhesion ability.

AroraNeha *et al*[30].evaluated the entrapment efficiency of glipizide microsphere. Glipizidemicrosphere was prepared by solvent evaporation technique using Eudragit RS 100 as a matrix polymer with dibutyl phthalate and diethyl phthalate as plasticizer and also without plasticizer. Increased in concentration of plasticizers the percentage entrapment efficiency of glipized was increased. Furthermore these microspheres can be orally administrated using capsule as a dosage form which will be ideal to maintain sustained release of glipizide.

Senthil A *et al*[31].investigated the design and characterization of mucoadhesive glipizide microsphere using carbopol 974 as polymer. Microspheres were prepared by simple emulsification phase separation technique using glutaraldehyde as a crosslinking agent. From twenty preliminary trail batches the optimized formulation was selected based on their percentage of mucoadhesion and sphericity of microspheres.3^2 full factorial design were employed.

Mishra Manoj kumar *et al*[32].prepared sustained-release ketorolac tromethamine microspheres of bovin serum albumin in different ratios by the emulsion cross-linking method using epichlorohdrin. The prepared microspheres were subjected to various physicochemical evaluation and in vitro release studies. The drug release from microspheres of 1:5 ratios is the most constant and prolonged drug release id diffusion followed by erosion.

Vikas Parashar *et al*[33].,developed biodegradable microspheres of Tinidazole using Bovine Serum Albumin. Four batches of Tinidazole microsphere were prepared by emulsion cross-linking method. The quantity of BSA varies for each formulation. Formulations were evaluated for particle size, Melting point, TLC, entrapment efficiency and *in vitro* release studies. The surface topography and internal textures of the microspheres was observed by scanning electron microscopy. The microspheres were spherical, discrete and compact and size distribution was between 33.28 to 36.25 μm. *In vitro* studies were carried out at different pH for a period of 18 h and compared with marketed formulation. From all the batches it is concluded that when concentration of polymer increases microspheres shows more controlled and

prolonged release. The drug release was between 66, 51, 48, 42 (in %). The drug release from 1:4 is most prolonged and constant.

AshviniUrs. V *et al*[34].designed and formulated ketoprofen loaded albumin microspheres by solvent evaporation technique. The microspheres were found to have incorporation efficiency of 48% to 79%. The effect of albumin concentration was evaluated with respect to entrapment efficiency, particle size, surface characterization and *in vitro* release behaviours. From the preliminary trials it was concluded that it is possible to formulate sustained release ketoprofen loaded albumin microspheres.

Aydan Gulsu *et al*[35].developed albumin microspheres of ketoprofen were prepared by emulsion polymerization method using glutaraldehyde as cross linking agent. Optimal conditions were determined as 0.1mg/ml of albumin concentration, 1000rpmstrring rate, 1% gultaraldehyde amount and 30 min crosslinking time.

China Gangadhar B *et al*[36].formulated and evaluated indomethacin microspheres using natural polymer, Egg albumin; semi synthetic polymer, Ethyl cellulose and synthetic polymer, meth acrylic acid esters (EudragitL 100) as the retardant materials. Microspheres were prepared by solvent evaporation method using an acetone / liquid paraffin system and Phase separation co-acervation method using petroleum ether and coconut oil as dispersion and continuous phase systems. The prepared microspheres were evaluated for their micromertic properties, drug content and encapsulation efficiency and characterized by Fourier transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM) and in vitro release. The release of Indomethacin was influenced by the drug to polymer ratio and particle size &was found to be both diffusion and dissolution controlled.

Sayyed Abolghas Semsajadi **Tabassi *et al*[37].**prepared bovine serum albumin based microspheres bearing propranolol hydrochloride by emulsion-internal phase stabilization technique. The drug release from albumin microspheres was mainly controlled by diffusion. The total amount of drug released from microspheres after 12h was 70%. In vitro experiments on the rat intestinal segments revealed that the microspheres could effectively pass their content through intestinal membrane.

Yamini Pendyala *et al*[38].formulated and systemically evaluated in vitro and in vivo performance of mucoadhesive Ramipril microspheres for its potential use in the treatment of hypertension, myocardial infraction. Ramipril mucoadhesive microspheres , containing chitosan as mucoadhesive polymer and ethylcellulose as varrier polymer , were prepared by an emulsion-solvent evaporation technique.F_4 showed good dissolution profile with 81.0%.

Dandagi **PM *et al* [39].**developed a bovine serum albumin (BSA) based microparticles bearing captropril were prepared by an emulsification-heat stabilization technique. The prepared microparticles were studied for drug loading, particle size distribution, *in vitro* release characterization, *in vivo* tissue distribution study and stability studies.

LITERATURE REVIEW OF GRANULES

Ravi S Wanare *et al*[40].enhanced dissolution rate of poorly water soluble drug by solid dispersion technique. Ramipril is a poorly water soluble drug and it also has poor bioavailability. the solid dispersion technique was evaluated for enhancement of solubility and dissolution rate. This study investigated the feasibility of quaternary solid dispersion of Ramipril with Poloxamer-188, HPMC and PVP K-30. The solid dispersions were characterized by FT-IR spectroscopy, differential scanning calorimetry and X-ray diffraction. The solubility and dissolution rate of solid dispersions were compared with untreated Ramipril.

Sudheer Nadendla *et al*[41].Performed preparation and evaluation of floating tablets of Ramipril as model drug. For prolongation of gastric residence time floating effervescent tablets were formulated by various materials like hydroxypropyl methylcellulose (HPMC) K4M, K 15M, K100M and micro crystalline cellulose and gas generating agent like sodium bicarbonate and evaluated for floating properties, swelling characteristics and drug release studies. In vitro drug release studies were performed and drug release kinetics evaluated using the linear regression method was found to follow both the Higuchi and the Korsemeyer Peppas equation. The drug release mechanism was found non -fickian type in most of the formulations.

The developed floating tablets of Ramipril may be used in clinic for prolonged drug release for at least 12 h, thereby improving the bioavailability and patient compliance.

Harish Chander *et al*[42].formulated and evaluated fast dissolving tablets of Ramipril using direct compression technique (effervescent) with sodium bicarbonate, mannitol, polyvinylpyrrolidone, citric acid. Eight different formulation of Ramipril were prepared by using different ratio of NAHCO: MANNITOL by direct compression method. The hardness, wetting time, water absorption ratio and wetting time ,in vitro release were also shows the satisfactory result. FDT4 batch with sodium bicarbonate: mannitol (1:3) showed more release than the other concentration and better results.

P Sivannarayana *et al*[43].designed sustained release matrix tablets of Ramipril using tamarind kernels mucilage and evaluate the effect of polymer on release pattern of the drug. Ramipril sustained release matrix tablets were prepared by wet granulation method by using starch as granulating agent. It was found that the release of drug from matrix tablet decrease with the increasing of percentage of polymer.

Rajesh A *et al*[44].worked to show the effect of various super disintegrates on the disintegration time and in vitro drug release rate on mouth dissolving tablets of ramipril. Disintegrating tablets are prepared by using different super disintegrants following wet granulation method. The Tablets were formulated by direct compression method, using Mannitol as diluent. Crospovidone (XL-PVP), croscarmellose sodium (Ac-Di-Sol®), The Precompression parameters and post compression parameters are performed. The optimized formulation was selected based on the results and stability studies were carried out on the optimized formulation and the percentage drug release was found to be 97.8%.

Sachin Patil *et al*[45]. Developed a stable and optimized bilayer tablet having extended release (ER) layer of Metoprolol Succinate which is a Beta blocker and immediate release(IR) layer of Ramipril which is an ACE inhibitor and successfully relievehypertention. Eight batches of bilayer tablets were developed by Direct Compression technique. FTIR and DSC were carried out. It was found that

the in vitro drug release of Metoprolol Succinate ER was best explain by Higuchi model. Formulation 7 complied with all the USP specifications and thus was taken as stability batch successfully.

Ratnaparkhi Mukesh *et al*[46]. Formulated and Evaluated Immediate Release Tablets of Metformin HCl and Glibenclemide Using Different Superdisintegrants (Sodium Starch Glycolate, Croscarmellose, Crospovidone and polacrinpotasium) and by wet granulation method. The drug-excipients interaction was investigated by FTIR. The granules and tablets of metformin and glibenclamide were evaluated for various pre and post compression parameters. Their results were found that the in vitro dissolution studies show the release is in the following order of superdisintegrants : Sodium Starch Glycolate >Crosspovidone>Polacrin potassium >Croscarmellose Sodium.

AIM AND PLAN OF WORK

AIM AND PLAN OF WORK

AIM OF THE WORK

Angiotensin-converting enzyme (ACE) inhibitors are the first line drugs for the treatment of Hypertension. Ramipril is a prodrug belonging to the ACE inhibitor class of medication. It is metabolized to ramiprilat in liver and to lesser extent to kidney. Ramiprilat is a potent, competitive inhibitor of ACE, the enzyme responsible for the conversion of angiotensin I to angiotensin II. Angiotensin II regulated blood pressure and is a key component of the rennin-angiotensin –aldosterone system (RAAS). Ramipril is used in the treatment of hypertension, congestive heart failure, nephropathy, and myocardial infraction.

The aim of the present study is

- To formulate and evaluate Pulsatile drug delivery system of ramipril microspheres and granules providing chronomodulated therapy for the better treatment of hypertension.
- To provide the drug at a time when it is needed most and dose related side effect could be minimized.

PLAN OF WORK

- Preformulation studies
- Calibration curve for Ramipril
- Preparation of Modified Pulsincap of Ramipril
    - Preparation of Immediate release granules
    - Preparation of cross-linked gelatin capsules
    - Preparation of Ramipril Microspheres
    - Preparation of polymer plug
    - Formulation of modified pulsincap

- Evaluation
    - Angle of repose
    - Compressibility index
    - Hausner's ratio
    - Drug content uniformity
    - Test for formaldehyde treated empty capsules
    - Physiochemical characterization of hydrogel plug
    - Determination of swelling index of hydrogel plug
    - Particle size determination
    - Scanning Electron Microscopy (SEM)
    - Percentage yield
    - Drug loading capacity
    - *In-vitro* release of Ramipril IR
    - *In-vitro* release of Ramipril Microspheres
    - *In-vitro* release of Ramipril Pulsincap
    - Release kinetics of optimized formulation
    - Stability of optimized formulation as per ICH guidelines

RATIONAL OF STUDY

RATIONALE OF THE STUDY

The rational of this study was to design and evaluate an oral site specific pulsatile drug delivery system containing Ramipril which can be targeted in a time dependent manner to modulate the drug level in synchrony with the circardian rhythm of congestive heart failure.

In this research work an attempt was made to develop a novel dosage form using a chronopharmaceutical approach.

Ramipril is a prodrug belonging to the angiotensin-converting enzyme (ACE) inhibitor class of medications. It is metabolized to ramiprilat in the liver and, to a lesser extent in kidneys. Ramiprilat is a potent, competitive inhibitor of ACE, the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensin-aldosterone system (RAAS).

RAMIPRIL IS SELECTED AS A MODEL DRUG FOR FOLLOWING REASONS

- Mainly used for the relief from hypertension, congestive heart failure, nephropathy and myocardical infraction.
- A shorter half-life (2-4hrs).
- Poorly water soluble drug which undergoes first pass metabolism.
- Absolute bioavailability of 28-35%

RATIONALE FOR SELECTION OF CHRONOMODULATED DOSAGE FORM

- Chronopharmacotherapy of diseases which show circadian rhythms in their pathophysiology.
- Avoiding the first pass metabolism e.g. protein and peptides
- For drugs which exhibit tolerance,
- For targeting specific site in intestine e.g. colon,
- For time programmed administration of hormone and drugs
- For drugs having the short half life

DISEASE PROFILE

DISEASE PROFILE[47]

Hypertension is the medical term for high blood pressure. It is known as the "silent killer". Hypertension is high blood pressure. Blood pressure is the force of blood pushing against the walls of arteries as it flows through them. Arteries are the blood vessels that carry oxygenated blood from the heart to the blood's tissues.

Hypertension is the major health problem, because it has no symptoms. Hypertension is more common in men than women and in people over the age of 65 have hypertension. Hypertension is serious because people with the condition have a higher risk for heart disease and other medical problems than people with normal blood pressure. If left untreated, hypertension can lead to the following medical condition, arteriosclerosis (atherosclerosis), heart attack, stroke, enlarged heart, kidney damage.

TYPES OF HYPERTENSION

1. Essential Hypertension

Most patients with hypertension have essential hypertension (also known as primary hypertension), with no identifiable cause for their disorder.

2. Secondary Hypertension

Patients with secondary hypertension have a specific identified cause for elevated BP. Although only 5% to 10% of those among the hypertensive population have secondary hypertension Secondary causes are potentially correctable. Further diagnostic workup also should be considered in patients who do not respond to increasing doses of antihypertensive medication or who have a sudden increase in BP or accelerated or malignant hypertension. A thorough review of prescription medications, nonprescription medications, and supplements should be conducted to rule out potential causes of BP elevations.

3. Pseudohypertension

The possibility of pseudohypertension should be considered when measuring BP in elderly patients. In pseudohypertension, blood vessels become stiff and thick because of calcification and resist compression from the bladder of the inflatable BP cuff. Pseudohypertension is thought to be relatively rare.

4. White-Coat Hypertension

White-coat hypertension describes patients who have consistently elevated BP values measured in a clinical environment in the presence of a health care professional (e.g., physician's office), yet when measured elsewhere or with 24-hour ambulatory monitoring, BP is not elevated.

Clinically Hypertension is classified based on the systolic and diastolic blood pressure given in table.

Table 1 : Classification of Clinical Hypertension

Blood pressure Classification	Systolic (mmHg)	Diastolic (mmHg)
Normal	<120	And<80
Pre- hypertension	120-139	Or 80-90
Stage 1 hypertension	140-159	Or 90-99
Stage 2 hypertension	≥160	Or ≥100
Isolated systolic hypertension	≥140	<90

PATHOPHYSIOLOGY OF BP REGULATION

Various neural and humoral factors are known to influence and regulate BP. These include the adrenergic nervous system (controls α- and β-receptors), the renin-angiotensin-aldosterone system (RAAS) (regulates systemic and renal blood flow), renal function and renal blood flow (influences fluid and electrolyte balance), several hormonal factors (adrenal cortical hormones, vasopressin, thyroid hormone, insulin), and the vascular endothelium (regulates release of nitric oxide, bradykinin, prostacyclin, endothelin). BP is normally regulated by compensatory mechanisms that respond to changes in cardiac demand. An increase in cardiac output (CO) normally results in a compensatory decrease in total peripheral resistance (TPR); likewise, an increase in TPR results in a decrease in CO.

The kidney plays an important role in the regulation of arterial pressure, especially through the RAAS. Decreases in BP and renal blood flow, volume depletion or decreased sodium concentration, and an activation of the sympathetic nervous system can all trigger an increased secretion of the enzyme renin from the cells of the juxtaglomerular apparatus in the kidney. Renin acts on angiotensinogen to catalyze the formation of angiotensin-1. Angiotensin-converting enzyme (ACE) converts angiotensin-1 to angiotensin-2. Angiotensin-2 is a potent vasoconstrictor that acts directly on arteriolar smooth muscle and also stimulates the production of aldosterone by the adrenal glands. Aldosterone causes sodium and water retention and the excretion of potassium. Several factors influence renin release, especially those that alter renal perfusion.

Arterial BP also is regulated by the adrenergic nervous system, which causes contraction and relaxation of vascular smooth muscle. Stimulation of α-adrenergic receptors in the central nervous system (CNS) results in a reflex decrease in sympathetic outflow causing a decrease in BP. Stimulation of postsynaptic α_1-receptors in the periphery causes vasoconstriction. α-Receptors are regulated by a negative feedback system; as norepinephrine is released into the synaptic cleft and stimulates presynaptic α_2-receptors, further norepinephrine release is inhibited. This negative feedback results in a balance between vasoconstriction and vasodilatation.

Stimulation of postsynaptic β_1-receptors located in the myocardium causes an increase in heart rate and contractility, whereas stimulation of postsynaptic β_2-receptors in the arterioles and venules results in vasodilation.

Epidemiologic evidence and clinical trials have demonstrated an inverse relationship between calcium and BP. Increased intracellular calcium concentrations can increase peripheral vascular resistance, resulting in increased BP.

A decrease in potassium has been associated with an increase in peripheral vascular resistance.Insulin resistance and hyperinsulinemia also have been associated with hypertension.

Angiotensin-2 promotes vasoconstriction of the vascular epithelium. Several other substances regulate vascular tone, however. Nitric oxide (NO) is produced in the endothelium and is a potent vasodilatory chemical that relaxes the vascular epithelium. The NO system has been firmly established as an important regulator of arterial BP. Hypothetically, some patients with hypertension have an intrinsic deficiency in NO release and inadequate vasodilation, which could contribute to hypertension and its vascular complications.

FIRST-LINE AGENTS

Angiotensin Converting Enzyme Inhibitors (ACEIs)

The ACEIs directly inhibit angiotensin-converting enzyme and, therefore, block the conversion of angiotensin-1 to angiotensin-2. This action reduces angiotensin-2–mediated vasoconstriction and aldosterone secretion, and ultimately lowers BP. Because additional pathways exist for the formation of angiotensin-2, ACEIs do not completely block the production of angiotensin-2. These agents generally do not cause metabolic effects. Hyperkalemia is possible, however, and potassium concentrations should be monitored. Patients with chronic kidney disease or volume depletion may be more susceptible to hyperkalemia or to further kidney dysfunction. Bradykinin accumulates in some patients because inhibiting ACE prevents the breakdown and inactivation of bradykinin. Although this may lead to

additive vasodilation by releasing nitrous oxide, bradykinin can also cause a dry cough in some patients. Cough is the most frequent, yet harmless side effect of ACEI therapy.

Angiotensin Receptor Blockers (ARBs)

The newest antihypertensive agents are ARBs. They modulate the RAAS by directly blocking the angiotensin-2 type 1 receptor site. Therefore, they block angiotensin-2–mediated vasoconstriction and aldosterone release. Overall, ARBs are very well tolerated. They do not affect bradykinin and, therefore, do not cause a dry cough as do the ACEI. Because aldosterone is blocked, monitoring of potassium is important to avoid hyperkalemia.

Calcium Channel Blockers (CCBs)

The CCBs are pharmacologically complex. They reduce calcium entry into smooth muscles, cause coronary and peripheral vasodilation, and lower BP. All decrease cardiac contractility (except amlodipine and felodipine). Dihydropyridine CCBs are primarily vasodilators that can cause a reflex tachycardia. Non-dihydropyridine CCBs (verapamil and diltiazem) directly block the AV node, decrease heart rate, decrease cardiac contraction, and have some vasodilatory effects. Side effects depend on the individual CCB used, but can include flushing, peripheral edema, tachycardia, bradycardia or heart block, and constipation.

Thiazide Diuretics

Diuretics, particularly thiazide diuretics, such as hydrochlorothiazide (HCTZ), have been extensively studied in large clinical trials for hypertension. When initially started, they induce a natriuresis that causes diuresis and decreases plasma volume. Diuresis usually decreases after chronic use with some of these agents, especially with thiazide diuretics. The long-term BP lowering effects are maintained because of a sustained decrease in peripheral vascular resistance (PVR).

SECOND-LINE AGENTS

β-Blockers

β-Blockers have several direct effects on the CV system. They can decrease cardiac contractility and output, lower heart rate, blunt sympathetic reflex with exercise, reduce central release of adrenergic substances, inhibit norepinephrine release peripherally, and decrease renin release from the kidney. All these contribute to their antihypertensive effects. Adverse metabolic effects include altered lipids and increased glucose concentrations. Similar to diuretics, these changes are generally temporary, however, and have minimal to no clinical significance. In primary prevention patients, they should be used as add-on therapy in combination with the other first-line agents (ACEI, ARB, CCB, or thiazide diuretic).

Aldosterone Antagonists

Spironolactone and eplerenone are aldosterone antagonists, also classified as potassium-sparing diuretics. Potent blockade of the aldosterone receptor inhibits sodium and water retention, and inhibits vasoconstriction. Hyperkalemia, a known dose-dependent effect.

DRUG PROFILE

DRUG PROFILE[48,51]

RAMIPRIL

Category

Antihypertensive Agents, ACE (Angiotensin converting enzyme) inhibitors

Physiochemical Properties

Description : A white or almost white, crystalline powder.

Solubility : Freely soluble in Methanol, Sparingly soluble in water

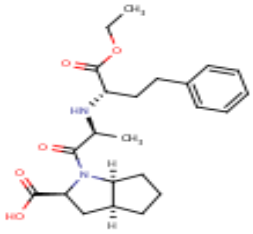
CAS Number : 87333-19-5

Chemical Name :(2S,3aS,6aS)-1-[(S)-2-[[(S)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]

Molecular Formula : $C_{23}H_{32}N_2O_5$

Molecular Weight : 416.5106

Structural Formula :



MECHANISM OF ACTION

Ramipril is a prodrug belonging to the ACE inhibitor class of medication. Ramiprilat, the principle active metabolite of ramipril, competitive inhibitor of Angiotensin converting enzyme, the enzyme responsible for the conversion of angiotensin I(ATI) to angiotensin II (ATII). AT II regulates blood pressure and is a key component of the rennin-angiotensin-aldosterone system (RAAS). Ramipril also causes an increase in plasma renin activity likely due to a loss of feedback

inhibition mediated by AT II on the release of renin and/or stimulation of reflex mechanisms via baroreceptors.

PHARMACOKINETICS

Absorption

The extent of absporption is at least 50-60%. Food decreases the rate of absorption from the GIT without affecting the extent of absorption. The absolute bioavailability of ramipril and ramiprilat were 28 and 44% respectively, when oral administration was compared to intravenous administration.

Protein Binding

Protein binding of ramipril is about 73% and that of ramiprilat about 56%

Half- life : 2-4 hrs

CLINICAL APPLICATION

The indications are Hypertension ,Congestive heart failure, Myocardial infarction, Diabetic Nephropathy

TOXICITY

Symptoms of overdose may include excessive peripheral vasodilation (with marked hypotension and shock), bradycardia, electrolyte disturbances, and renal failure. The most likely adverse reactions are symptoms attributable to its blood-pressure lowering effect. May cause headache, dizziness, asthenia, chest pain, nausea, peripheral edema, somnolence, impotence, rash, arthritis, and dyspnea.

DOSAGE

a) **Initial** – 2.5mg once daily increased to 5mg once daily after 1 week if tolerated.
b) **Maintenance** – 10mg one daily after a further three week.

EXCIPIENT PROFILE

EXCIPIENTS PROFILE

ALBUMEN[50]

1. Nonproprietary Names

BP: Albumin Solution

PhEur: Human Albumin Solution

USP: Albumin Human

2. Synonyms

Alba; Albuconn; Albuminar; albumin human solution; albumin humanisolutio; Albumisol; Albuspan; Albutein; Buminate; human serum albumin; normal human serum albumin; Octalbin; Plasbu-min; plasma albumin; Pro-Bumin ; Proserum; Zenalb.

3. Chemical Name and CAS Registry Number

Egg albumin [9006-59-1]

4. Empirical Formula and Molecular Weight

Human serum albumin has a molecular weight of about 66 500 andis a single polypeptide chain consisting of 585 amino acids.

5. Functional Category

Stabilizing agent; therapeutic agent.

6. Applications in Pharmaceutical Formulation or Technology

Albumin is primarily used as an excipient in parenteral pharmaceutical formulations, where it is used as a stabilizing agent for formulations containing proteins and enzymes.

Albumin has also been used to prepare microspheres and microcapsules for experimental drug-delivery systems.

As a stabilizing agent, albumin has been employed in protein formulations at concentrations as low as 0.003%, although concentrations of 1–5% are commonly used. Albumin has also been used as a cosolvent for parenteral drugs, as a cryoprotectant during lyophilization, and to prevent adsorption of other proteins to surfaces. Therapeutically, albumin solutions have been used parenterally for plasma volume replacement and to treat severe acute albumin loss. However, the benefits of using albumin in such applications in critically ill patients have been questioned.

7. Description

Albumin human as a sterile nonpyrogenic preparation of serum albumin obtained from healthy human donors; see Section 13. It is available as a solution containing 4, 5, 20, or 25 g of serum albumin in 100 mL of solution, with not less than 96% of the total protein content as albumin. The solution contains no added antimicrobial preservative but may contain sodium acetyltryptophanate with or without sodium caprylate as a stabilizing agent. In the solid state, albumin appears as brownish amorphous lumps, scales, or powder.

8. Solubility

Freely soluble in dilute salt solutions and water. Aqueous solutions containing 40% w/v albumin can be readily prepared at pH 7.4. The high net charge of the peptide contributes to its solubility in aqueous media. The seven disulfide bridges contribute to its chemical and spatial conformation.

9. Stability and Storage Conditions

Albumin is a protein and is therefore susceptible to chemical degradation and denaturation by exposure to extremes of pH, high salt concentrations, heat, enzymes, organic solvents, and other chemical agents. Albumin solutions should be protected from light and stored at a temperature of 2–25 8 C or as indicated on the label.

HYDROXYL PROPYL METHYL CELLULOSE[50]

1. Non Proprietary Names

BP :Hydromellose

JP : Hydroxyl propyl methyl cellulose

PhEur :Hypomellosum

USP- NF :Hypromellose

2. **Synonyms**

Benece, MHPC; E464; hydroxyl propyl methylcellulose; HPMC: Methocel; methylcellulose propylene glycol ether; methyl hydroxyl propyl cellulose; Metolose; Tylopur.

3. Chemical Name

Cellulose hydroxyl propyl methyl ether.

4. CAS Registry Number

[9004-65-3]

5. Molecular Formula

Approximately 10000-1500000.

6. Functional Category

Bioadhesive material, dissolution enhancer, controlled release agent, modified release agent, solubilizing agent, mucoadhesive, coating agent, film-former, rate-controlling controlling polymer for sustained release, stabilizing agent, suspending agent, tablet binder, viscosity- increasing agent.

7. Applications in Pharmaceutical Formulation

- Hypromelloseis widely used in oral, ophthalmic, nasal, and topical pharmaceutical formulations
- Concentrations between 2% to 5% w/w may be used as a binder in either wet or dry granulation process.
- High- viscosity grades may be used to retard the release of drugs from a matrix at levels of 10-80% w/w tablet and capsules.

- Hypromelloseis also used in liquid oral dosage forms as a suspending and/or thickening agent at concentrations ranging from 0.25-5.0%

8. Description

Hypromelloseis an odourless and tasteless, white or creamy-white fibrous or granular powder.

9. Solubility

Soluble in cold water, forming a viscous colloidal solution; practically insoluble in chloroform, ethanol (95%), and ether, but soluble in mixtures of ethanol and dichloromethane, mixtures of methonal and dichloromethane, and mixtures of water and alcohol.

10. Incompatibility

Incompatible with some oxidizing agents. Since it is non-toxic, hypromellose will not complex with metallic salts or ionic organincs to form insoluble precipitates.

11. Stability

Hypremellose powder is a stable material, although it is hygroscopic after drying. It should be stored in a well-closed container, in a cool, dry place.

12. Safety

Hypromelloseis generally regarded as a nontoxic and non-irritant material, although excessive oral consumption may have a laxative effect.

EXCIPIENTS PROFILE

MICROCRYSTALLINE CELLULOSE[50]

1. Non- Proprietary Names

BP : Microcrystalline cellulose

JP : Microcrystalline cellulose

PhEur :Cellulosummicrocristallinum

USP-NF : Microcrystalline cellulose

2. Synonyms

Avicel PH, celex, cellulose gel, celphere, ceolus KG, crystalline cellulose, E460, Emcocel, Ethispheres, Fibrocel, Pharmacel, Tabulose, Vivapur.

3. Chemical Name

Cellulose

4. CAS Registry Number

[9004-34-6]

5. Empirical Formula

$[C_6H_{10}0_5]_n$ where n=220

6. Molecular Weight

36000

7. Functional Category

- Adsorbent
- Suspending agent
- Tablet and capsule diluent
- Tablet disintegrant

8. Applications in Pharmaceutical Formulation

- As a binder/diluents in oral tablet and capsule formulation in both wet-granulation and direct- compression process.
- Its use as a binder/diluents, microcrystalline cellulose also has some lubricant and disintegrant properties that make it useful in tableting.
- Microcrystalline cellulose is also used in cosmetics and food products.

9. Solubility

Slightly soluble in 5% w/v sodium hydroxide solution, practically insoluble in water, dilute axcids, and most organic solvents.

10. Description

Microcrystalline cellulose is a purified, partially depolymerizerd cellulose that occurs as a white, odourless, tasteless, crystalline powder composed of porous particles. It is commercially available in different particle sizes and moisture grades that have different properties and applications.

11. Incompatibilities

Cellulose acetate is incompatible with strongly acidic or alkaline substance. Cellulose acetate is compatible with the following plasticizers, diethyl phthalate, polyethylene glycol, triacetin, and triethyl citrate.

12. Stability and storage conditions

Microcrystalline cellulose is a stable through hygroscopic material. The bulk material should be stored in a well- closed container in a cool, dry place.

MATERIALS AND METHODS

MATERIALS USED

To formulate the Ramipril Pulsincap, the following materials were obtained from manufacturers and used.

Table 2: Drugs and Excipients

S.No	Drug/Exicipents	Manufacturer/ Supplier	Use in formulation
1.	Ramipril	Vee care	Active Pharmaceutical Ingredient
2.	HPMCK 4M	MMC Health Care	Hydrogel plug
3.	Egg Albumin	Fisher Scientific	Polymer
4.	Microcrystalline cellulose	Pharma French Ltd	Diluent
5.	PVPK30	Pharma French Ltd	Binding agent
6.	Sodium starch glycolate	Pharma French Ltd	Super disintegrant
7.	Liquid paraffin	Microfine Chemicals	Dispersion Medium
8.	n-hexane	Microfine Chemicals	Solvent
9.	Span- 80	Orgochem	Stabilizing agent
10.	Glutaraldehyde	Merck Lab	Crosslinking agent
11.	Isopropyl alcohol	Bafna , chennai	Solvent

INSTRUMENTS AND EQUIPMENTS

To perform the formulation and analysis of Ramipril pulsatile drug delivery, the following instruments/equipments were utilized.

Table 3: Instruments And Equipments

S.No	Instruments/ Equipments	Manufacturer/ Suppliers
1.	Electronic Weighing Balance	Asha scientific company, Mumbai.
2.	Magnetic Stirrer	Remi Equipment
3.	Hot air oven	MC Dalal, Chennai
4.	8 Station compression machine	Rimek, India
5.	pHmeter	MC Dalal, Chennai
6.	Dissolution tester	Veego, India
7.	Vernier Caliper	Mitutoyo, Japan
8.	UV-visible spectrophotometer	Shimadzu, Japan
9.	Fourier transform infra-red spectrophotometer	Nicolet, India
10.	Microscope	Sigma scientific instrumentation, Chennai.
11.	Scanning Electron Microscopy	Hitachi

METHODOLOGY

PREFORMULATION STUDIES[51]

Preformulation testing is the first step in the rationale development of dosage forms of a drug substance. It is defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms, which can be mass-produced.

Following preformulation studies were preformed,

1. Identification of Pure Drug

Identification of Ramipril was carried out by Infrared Spectroscopy.

2. Drug-Excipient Compatibility Studies

A successful formulation of a stable and effective solid dosage form depends on careful selection of excipients that are added to facilitate administration, promote the consistent release and bioavailability of the drug and protect it from degradation. If the excipients are new and not been used in formulation containing the active substance, the compatibility studies are of paramount importance.

Compatibility of Ramipril with the respective polymers and excipients that is Egg albumin, sodium starch glycolate, microcrystalline cellulose, polyvinyl pyrollidone and the physical mixture of main formulation was established by infrared Absorption Spectra Analysis(FTIR). Any changes in the chemical composition after combining with the excipients were investigation with IR spectras.

MATERIALS AND METHODS

ANALYTICAL METHODS[44,52]

Standard Calibration Curve of Ramipril

Calibration curve of Ramipril was prepared in three different buffers i.e. in 0.1N Hydrochloric acid, phosphate buffer pH 6.8 and phosphate buffer pH 7.4.

Preparation of 0.1 N Hydrochloric acid

0.1 N Hydrochloric acid was prepared by diluting 8.5 ml of concentrated Hydrochloric acid to 1000ml with distilled water.

Preparation of Phosphate Buffer pH 6.8

27.22 g of monobasic potassium phosphate was weighed and dissolved in 1000ml of distilled water to get stock solution of potassium phosphate. 8g sodium hydroxide was weighed and dissolved in 1000ml of distilled water to get 0.2M sodium hydroxide solution. 50ml of the monobasic potassium phosphate solution and 22.4 ml of sodium hydroxide solution were mixed and made up to 2000ml with distilled water.

Preparation of Phosphate Buffer pH 7.4

27.22g of monobasic potassium phosphate was weighed and dissolved in 1000ml of distilled water to get stock solution of monobasic potassium phosphate. 8g of sodium hydroxide was weighed and dissolved in 1000ml distilled water to get 0.2M sodium hydroxide solution.50ml of the monobasic potassium phosphate solution and 39.1ml of 0.2M sodium hydroxide solution were mixed and made upto 2000ml with distilled water.

Calibration curve of Ramipril

A stock solution was prepared by adding 10mg of drug in 100ml of0.1 N Hydrochloric acid,phosphate buffer pH6.8 and phosphate buffer pH 7.4 separately. The above solution was serially diluted with respective buffers to obtain solutionsin the concentration in the range of 10-50µg/ml. The absorbance of the samples was measured.

Preparation of Immediate Release Granules[53]

Table:4 Formulation of Immediate Release Granules

S.No	Ingredient	G1(mg)	G2(mg)	G3(mg)	G4(mg)
1	Ramipril	**500**	**500**	**500**	**500**
2	Sodium starch glycolate	**86**	**178**	**272**	**386**
3	Microcrystalline cellulose	**180**	**180**	**180**	**180**
4	PVP K30	**3.6**	**3.6**	**3.6**	**3.6**

Granules of Ramipril were made by wet granulation method. Ramipril, Sodium starch glycolate, Microcrystalline cellulose were weighed accurately and blended homogeneously for 15 minutes. Polyvinyl pyrollidone was dissolved in isopropyl alcohol and mixed with the powder blend to get a coherent mass. The mass was passed through sieve no 22.

EVALUATION OF GRANULES

Precompression Studies[20, 51]

1. **Angle of Repose**

The angle of repose of blend was determined by the funnel method. The accurately weighted blend was taken in the funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the blend. The blend was allowed to flow through the funnel freely on to the surface. The diameter of the

powder cone was measured and angle of repose was calculated using the following equation.

tan Ø= h/r

Where, h and r are the height of the powder cone.

2. Bulk Density

Density is defined as weight/unit volume. Bulk density is defined as the mass of the powder divided by the bulk volume and is expressed as g/cm^3 .The bulk density of a powder primarily depends on particle size distribution, particle shape and tendency of particles to adhere together. Bulk density was determined by flowing powder into a graduated cylinder. The bulk volume and weight of the powder were determined .The bulk density was calculated using the following formula,

Bulk density = mass of the powder /bulk volume

3. Tapped Density

It is defined as the ratio of total mass of the powder to the tapped volume of the powder. Tapped density was determined by pouring gently a specified quantity of sample through a glass funnel into a 50ml graduated cylinder. The cylinder was tapped from a height of 2 inches until a constant volume was obtained. Tapped density was measured after 300 taps. Volume occupied by the sample after tapping was recorded and tapped density was calculated by the following formula,

Tapped density =mass of the powder/tapped volume

4. Carr's Compressibility Index (CI)

Compressibility is the ability of the powder to decrease in volume under pressure. Compressibility is the measure that is obtained from density determinations. The CI was calculated using the formula,

CI = tapped density –bulk density / tapped density ×100

5. Hausner's Ratio (HR)

It was determined by the ratio of tapped density to bulk density. It is calculated by the following formula,

HR= tapped density/bulk density

Table:6 Angle Of Repose, Compressibility Index. Hausner's Ratio

Flow property	Angle of repose	Compressibility Index	Hausner's Ratio
Excellent	25-30	<10	1-1.1
Good	31-35	11-15	1.12-1.18
Fair	36-40	16-20	1.19-1.25
Passable	41-45	21-25	1.26-1.34
Poor	46-55	26-31	1.35-1.45
Very poor	56-55	32-37	1.46-1.59
Very very poor	>66	>38	>1.60

6. Drug Content[53]

10mg granules were dissolved in a small quantity of methanol and the volume was made up to 100ml withphosphatae buffer pH 7.4. It was stirred for 12hrs. After stirring the solution was filtered through whatman filter paper and the absorbance was measured spectrophotometrically at 210nm after suitable dilution.

Preparation of Ramipril Albumin Microspheres[13]

Table: 5 Formulation of Microspheres

S.No.	Drug (mg)	Polymer (mg)
F1	500	500
F2	500	1000
F3	500	1250
F4	500	1500
F5	500	2000

Ramipril albumin microspheres were prepared by single emulsion polymerization technique. 100ml of liquid paraffin was taken in a beaker, mixed with 0.4% w/v span 60, Stirred and heated at 70ºC. The mixture was cooled to room temperature. Drug and polymer were dissolved in methanol and phosphate buffer respectively and mixed together. This mixture was then poured drop wise to liquid paraffin using hypodermic syringe with continuous stirring at 600 Rpm and 0.25ml of glutaraldehyde was added. It was mixed for 3 hours. Microspheres were separated by decantation, washed 6 times with petroleum ether and dried at room temperature. The microspheres were stored in a dessicator.

Preparation of Cross- Linked Gelatin Capsules[21]

The "1" sized hard gelatin capsules about 100 in number were taken. The body of the capsules was placed on a wire mesh. 25ml of 15%v/v formaldehyde was taken into a desiccator and potassium permanganate was added to it to generate formalin vapours. The wire mesh along with the body was kept in the dessicator. The reaction was carried out for 12 hours, after which the body were removed and dried at 50ºC for 30 minutes to ensure completion of reaction between gelatin and formaldehyde vapour. They were dried at room temperature to facilitate removal of residual formaldehyde.

Preparation of Hydrogel Plug

Plug for sealing the capsule body was prepared by compressing HPMCK4M granules in polyvinyl pyrollidoneusing 9mm punches on rotary tablet press.

Designing of Pulsincap

The pulsincap was similar in appearance to a hard gelatin capsules, but the body was water insoluble. Microspheres equivalent to 2.5mg of Ramipril were accurately weighed and filled into the formaldehyde treated body. The capsules containing the microspheres were plugged with prepared hydrogel plug and capsule cap was filled with Ramipril granules equivalent to 2.5mg and placed over the body.

EVALUATION OF MICROSPHERES [36,40]

1. **Drug-Polymer interaction Study**

Interaction between drug-polymer was studied by InfraRed spectroscopy using FTIR spectrometer. Sample preparation involved mixing the sample with potassium bromide (KBr), triturating in glass mortar and finally placing in the sample holder. The spectrum was scanned over a frequency range of 4000-400cm^{-1}

2. **Particle Size Analysis**

The size was measured using an optical microscope and the mean particle size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer.

3. **Angle of repose**

The angle of repose of blend was determined by the funnel method. The accurately weighted blend was taken in the funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the blend. The blend was allowed to flow through the funnel freely on to the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation.

$$\tan Ø = h/r$$

4. Drug content

10mg drug loaded microspheres were dissolved in a small quantity of methanol and the volume was made up to 100ml with phosphatae buffer pH 7.4. It was stirred for 12hrs. After stirring the solution was filtered through whatman filter paper and the absorbance was measured spectrophotometrically at 210nm after suitable dilution

5. Drug Loading capacity

Drug loading capacity was calculated by

Drug loading (%) = M actual / weighed quantity of powder of microspheres × 100

Where M actual is the actual drug content in weighed quantity of powder of microspheres

6. Percentage yield

The prepared microspheres were collected and weighed. The yield was calculated by dividing the measured weight by the total weight of all non-volatile components. The percentage yield of microspheres was calculated as follows.

$$\% \text{ Yield} = \frac{\text{Weight of microsphere}}{\text{Theoretical weight of drug and polymer}} \times 100$$

7. Scanning Electron Microscopy

The samples were dried thoroughly in vacuum desiccators before mounting on brass specimen studies. The samples were mounted on specimen studies using double sided adhesive type, and gold palladium alloy of 120A^{o} knees was coated on the sample using sputter coating unit in Argon ambient of 8 – 10 Pascal with plasma voltage about 20MA. The sputtering was done for nearly 3mins to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage of about 80MA. The condenser lens position was maintained between 4.4-5.1.

Physicochemical characterization of Hydrogel Plug[21]

Hydrogel Plugs were studied for hardness, friability, weight variation, lag time and Swelling Index.

Determination of Swelling Index of Hydrogel Plug

Hydrogel plugs were kept immersed in three different pH conditions. Plugs were taken out carefully at 2,4,6,8,10,12 hours and their weights were determined accurately.

$$\% \text{ Swelling} = \frac{\text{Wet weight} - \text{dry weight}}{\text{Wet weight}} \times 100$$

Evaluation of cross linked Empty Capsules

Various physical and chemical tests were carried out for formaldehyde treated and untreated capsules[21].

Physical tests

- **Identification**

  The capsules were observed physically.

- **Solubility Test for Formaldehyde Treated Capsules**

  The empty hard gelatin capsule was stirred vigorously in 100ml dissolution medium taken in 250ml beaker, with magnetic stirrer. Water, 0.1 N Hydrochloric acid1.2 pH, phosphate buffer pH 7.4 and phosphate buffer pH 6.8. The time at which the capsule dissolves or forms a soft mass was noted.

- **Dimension**

  Variation in dimension between formaldehyde treated and untreated capsules were studied. The length and diameter of capsules were measured before and after formaldehyde treatment, using caliper.

Chemical Test

Qualitative Chemical Test for Free Formaldehyde [7]

Formaldehyde solution (0.0002%w/v)was used as a standard solution. A sample solution was prepared by cutting 25 formaldehyde treated body of the capsules were cut into small pieces and placed in distilled water. This was stirred for 1hr with a magnetic stirrer, to solubilize the free formaldehyde. The solution was then filtered into a 50ml volumetric flask, washed with distilled water and the volume made up with 50 ml with the washings. To 1ml of sample solution, 9ml of water was added. 1ml of the resulting solution was mixed with 4ml of water and 5ml of acetone. The solution was warmed in a water bath at 40ºc and allowed to stand for 4 minutes.

***INVITRO* DISSOLUTION STUDIES**

a. For Ramipril Immediate release granules

The *in vitro* dissolution was carried out using USP Type I (Basket) dissolution apparatus under sink condition. The dissolution medium was 900 ml of a 0.1N HCl solution (pH=1.2), at $37^0C \pm 0.2^0C$ and the stirring speed of 50 rpm. The *in vitro* release studies were carried out for 2 hours.10ml of sample was taken at 10 minutes intervals for 2 hours and were replaced with fresh dissolution medium. The absorbance of the solution was recorded at 210 nm using UV spectrophotometer.

b. For Ramipril Microspheres

The *in vitro* dissolution was carried out using USP Type I (Basket) dissolution apparatus under sink condition. The dissolution medium was 900 ml of a phosphate buffer pH 6.8 at $37^0C \pm 0.2^0C$ and the rotating speed was 50 rpm. 10ml of sample was taken at 1hour intervals for subsequent hours and were replaced with fresh dissolution medium. The absorbance of the solution was recorded at 210 nm using UV spectrophotometer.

c. *In Vitro* Release of Pulsatile Capsule[21]

Dissolution studies were carried out using USP XXIII dissolution test apparatus (paddle method). Capsule was tied to paddle with a cotton thread so that the capsule was immersed completely in dissolution media but not float. In order to simulate the pH changes along the GI tract, three dissolution media with pH 1.2, 7.4 and 6.8 were sequentially used,(sequential pH change method). The pH 1.2 was first used for 2 hrs then removed and the fresh phosphate buffer pH 7.4was added. After 3 hrs the medium was removed and colonic fluid phosphate buffer pH 6.8 was added for subsequent study. Nine hundred milliliters of the dissolution medium was used at each time. Rotation speed was 100 rpm and temperature was maintained at 37±0.5 ten millilitres of dissolution medium was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were analysed at 210 nm, by UV absorption spectroscopy and the cumulative percentage release was calculated.

KINETIC ANALYSIS OF RAMIPRIL*IN VITRO* RELEASE DATA[40]

The *in vitro* drug release data were tested with the following mathematical model. The goodness of fit was found out to describe the kinetics of drug release.

Zero Order Release Model

Zero order models describe the systems where the drug release rate is independent of its concentration. The equation assumes that the cumulative amount of drug release is directly related to time.

$$C = k_0 t$$

Where,

C – Cumulative percentage drug release

$\mathbf{K_0}$ – Zero order rate constant expressed in unit concentration/time

t- Time in hour

A graph of concentration vs time would yield a straight line with a slope equal to k_0 and intercept the origin of x axis.

First Order Release Model

First order models describe the systems where the release rate is dependent on the concentration. The release behavior of first order equation is expressed as log cumulative percentage of drug remaining vs time. The equation as follows,

$$\mathbf{Log\ C = log\ C_o - kt / 2.303}$$

Where,

C- Cumulative percentage drug remaining

$\mathbf{C_o}$ – Initial concentration of drug

K – First order constant

A plot of time on x-axis and log cumulative percentage drug remaining on y-axis gives a straight line with slope, k/2.303 if it follows first-order kinetics.

Higuchi Square Root Law Model

The Higuchi model describes the release from systems where the solid drug is dispersed in an insoluble matrix and the rate of release is related to the rate of drug diffusion. This model describes the cumulative percentage of drug release vs square root of time. The equation is as follows,

$$\mathbf{Q = kt^{1/2}}$$

Where,

Q – Cumulative percentage drug released

K – Constant reflecting the design variables of the system

T – Time

A plot of square root of time on x-axis and cumulative percentage drug released on y-axis gives a straight line if it follows Higuchi Kinetics.

Hixson-Crowell Release Model

The Hixson-Crowell cube root model describes the release from systems where there is a change in surface area and diameter of the tablets or particles.

$$Q_0^{1/3} - Q_t^{1/3} = K_{HC}\, t$$

Where,

Q_t - Cumulative percentage drug released in time t,

Q_0 -Initial amount of drug

K_{HC} -Rate constant of Hixson-Crowell equation

A plot of time on x-axis and cube root of cumulative percentage of drug remaining on y-axis gives a straight line if it follows Hixson-Crowell kinetics.

Korsmeyer and Peppas Model

Korsmeyer and Peppas Model derive a simple relationship which describes the drug release from a polymeric system equation.

$$M_t / M_\infty = K\, t^n$$

Where,

M_t/M_∞ - Fraction of drug released at time t

k- Release rate constant

n - Release exponent

A plot of log time on x-axis and log cumulative percentage of drug released on y-axis gives a straight line if it follows Korsmeyer and Peppas kinetics. The n value is used to characterize different release mechanism.

Table: 7 Diffusion Exponent and Solute Release Mechanism for Cylindrical Shape

Diffusion exponent (n)	Overall solute diffusion mechanism
0.45	Fickian diffusion
0.45 <n<0.89	Anamolous (non-Fickian) diffusion
0.89	Case – II transport
n>0.89	Super case – II transport

STABILITY STUDIES[54]

Stability of a drug has been defined as the ability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic, and toxicological specifications. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light and enables recommended storage conditions, re-test periods and shelf lives to be established. The optimized formulation was selected and the stability study was carried out at accelerated condition of 40°C / 75% RH condition for a period of 3 months.

Method

Optimized formulation were packed in blister and stored in stability chambers maintained at 40°C / 75% RH for three months. After 3 months samples were analyzed for physical appearance, drug content and in vitro release.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

DRUG-EXCIPIENT COMPATIBILITY STUDY

The drug-excipient compatibility study was conducted to reveal the excipient compatibility with the drug.

Physical Compatibility Study: The physical compatibility of drug and excipients were given in Table 8.

Table 8: Physical Compatibility Study of Drug and Excipients

S.No.	Drug + Excipient	Description and Condition			
		Initial	Room Temperature and 40^0C/ 75% RH in Days		
			10^{th}	20^{th}	30^{th}
1	RAMIPRIL	White, almost white crystalline powder	NC	NC	NC
2	EGG ALBUMIN	Yellow powder	NC	NC	NC
3	HPMC K4M	White or creamy white crystalline powder	NC	NC	NC
4	MCC	White, crystalline powder	NC	NC	NC
5	PVPK30	White or Creamy white colored Hygroscopic Powder	NC	NC	NC
6	SSG	Creamy white Free flowing fine powder	NC	NC	NC
7	RAMIPRIL +EGG ALBUMIN	White, yellow colored powder	NC	NC	NC
8	RAMIPRIL + HPMC K4M	White or creamy white coloured powder	NC	NC	NC
9	RAMIPRIL +MCC	White, crystalline powder	NC	NC	NC
10	RAMIPRIL +PVP K30	White, crystalline powder	NC	NC	NC
11	RAMIPRIL + SSG	White or off white crystalline powder	NC	NC	NC

NC- No Change

The physical compatibility study was performed visually. The study implies that the drug and excipients were physically compatible with each other as there was no change of physical description. The excipients which were compatible with the drug were selected for formulation.

Chemical Compatibility Study (FTIR)

FTIR spectroscopy was carried out to study the compatibility of pure drug ramipril with the polymer albumin, and other excipients like microcrystalline cellulose, sodium starch glycolate, polyvinylpyrrolidone, hydroxyl propyl methylcellulose.

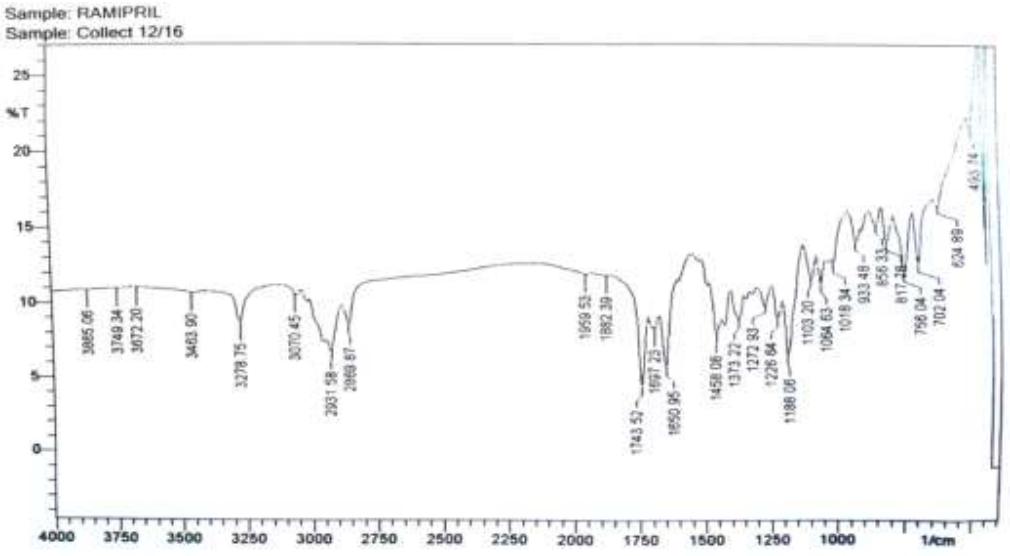


Figure 4. FTIR Spectra of Ramipril

Table 9. IR Interpretation of Ramipril

Wave Number (cm^{-1})	Interpretation
3672.20	OH stretching
3463.90	NH stretching
2869.87	CH stretching
1743.52	C=O ACID stretching
1650.95	C=O ESTER stretching

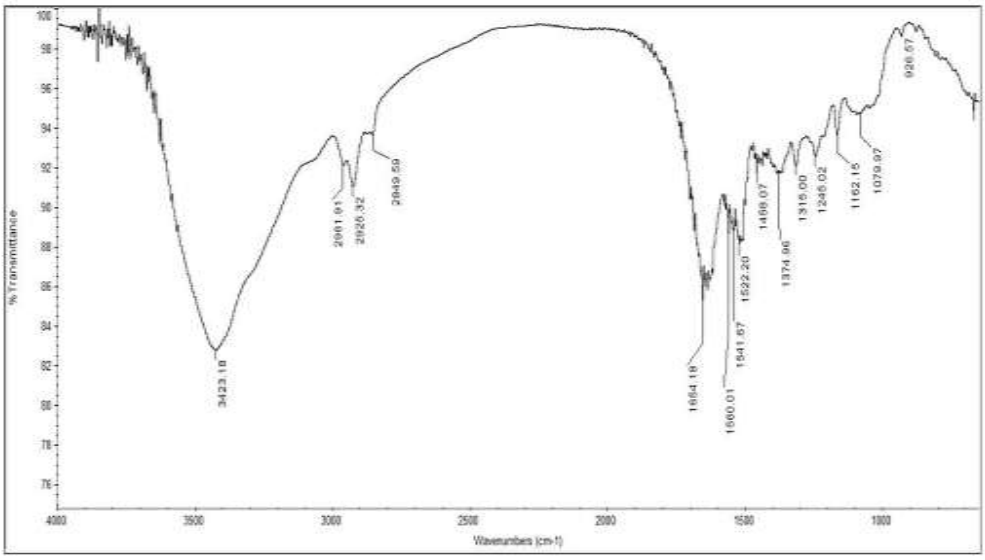


Figure5: IR of Egg Albumin

Table No :10 IR Interpretation of Egg Albumin

Wave length cm^{-1}	Interpretation
3423.18	O – H stretching
2961.91	C – H stretching
2925.32	C – H stretching
2849.59	C – H stretching
1654.18	C = N stretching
1560.01	C = O stretching
1541.67	C = O stretching

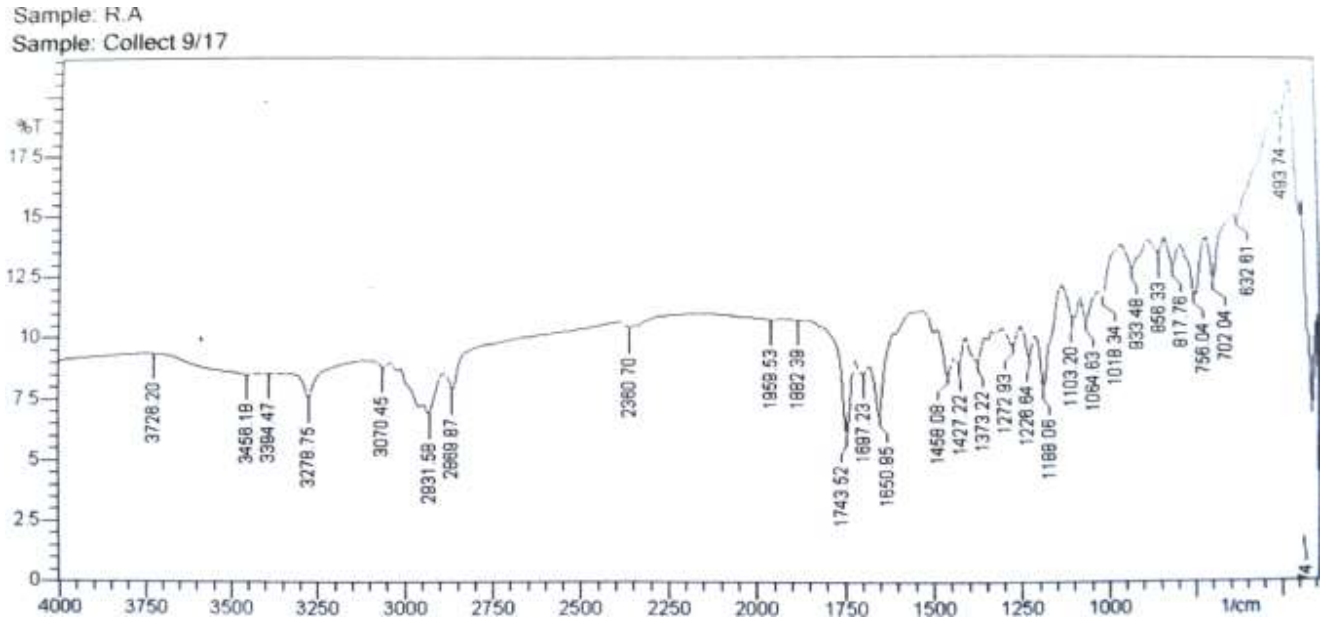


Figure6: FTIR of Ramipril and Egg Albumin

Table No :11 IR Interpretation of Egg Albumin and Ramipril

Wave length cm^{-1}	Interpretation
3423.18	O – H stretching
2961.91	C – H stretching
2925.32	C – H stretching
2849.59	C – H stretching
1654.18	C = N stretching
1560.01	C = O stretching
1541.67	C = O stretching

There is no appearance or disappearance of any characteristic peaks. This shows that there is no chemical interaction between the Ramipril and Egg Albumin

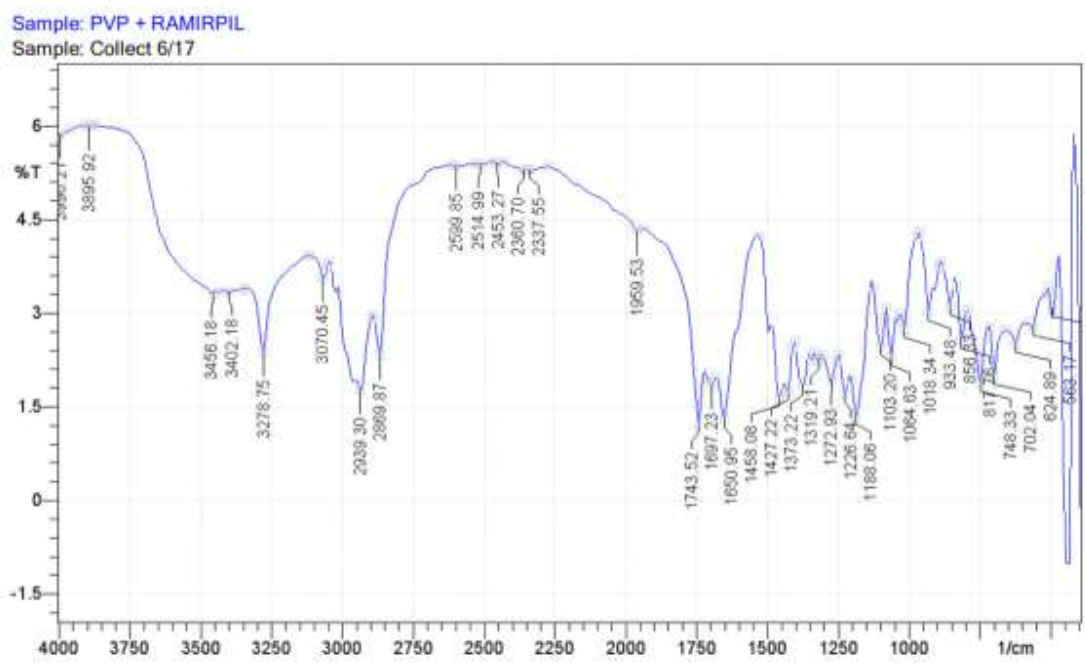


Figure 7. FTIR Spectra of Ramipril and PVP

Table 12. IR Interpretation of Ramipril and PVP

Wave Number (cm^{-1})	Interpretation
3885.92	OH stretching
3456.18	NH stretching
2869.30	CH stretching
1743.52	C=O ACID stretching
1650.95	C=O ESTER stretching

There is no appearance or disappearance of any characteristic peaks. This shows that there is no chemical interaction between the Ramipril and PVP.

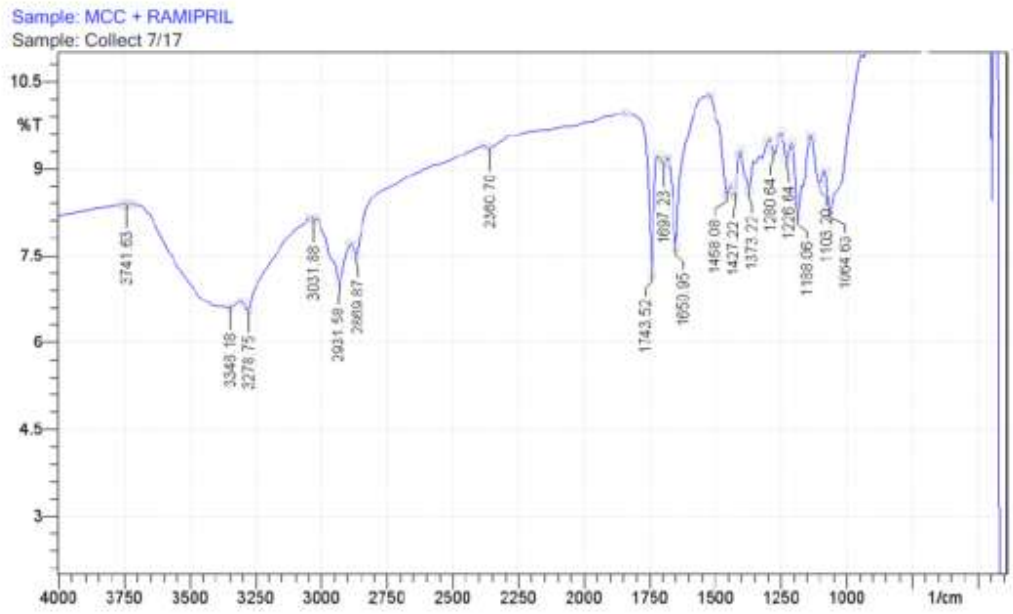


Figure 8. FTIR Spectra of Ramipril and MCC

Table 13. IR Interpretation of Ramipril and MCC

Wave Number (cm^{-1})	Interpretation
3741.63	OH stretching
3348.18	NH stretching
2869.87	CH stretching
1743.52	C=O ACID stretching
1650.95	C=O ESTER stretching

There is no appearance or disappearance of any characteristic peaks. This shows that there is no chemical interaction between the Ramipril and MCC.

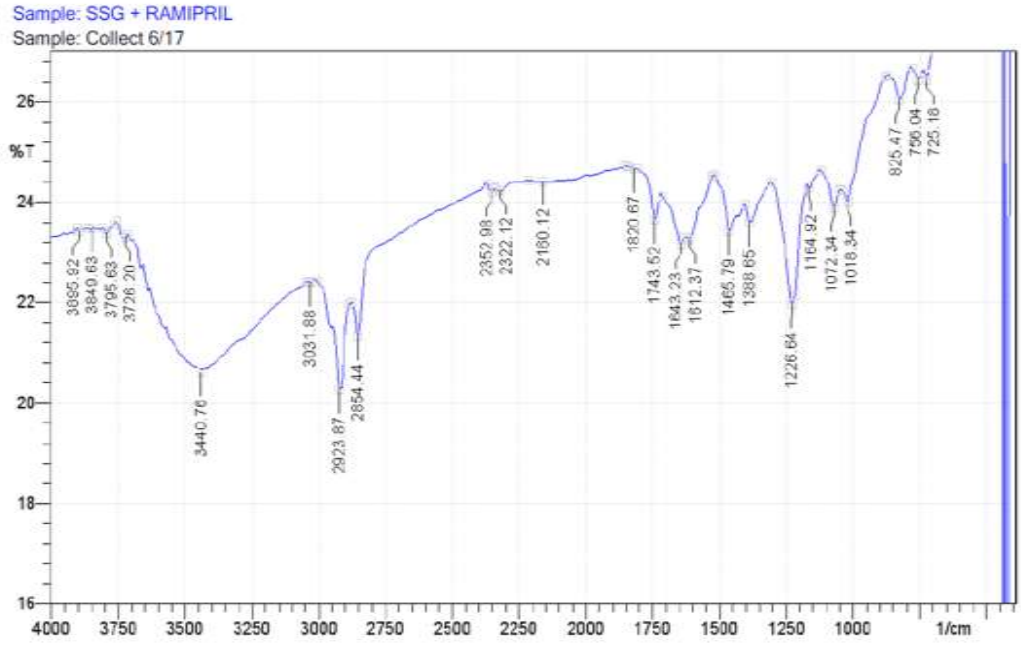


Figure 9. FTIR Spectra of Ramipril and SSG

Table 14. IR Interpretation of Ramipril and SSG

Wave Number (cm^{-1})	Interpretation
3795.63	OH stretching
3440.76	NH stretching
2923.87	CH stretching
1820.67	C=OACID stretching
1743.52	C=O ESTER stretching

There is no appearance or disappearance of any characteristic peaks. This shows that there is no chemical interaction between the Ramipril and SSG.

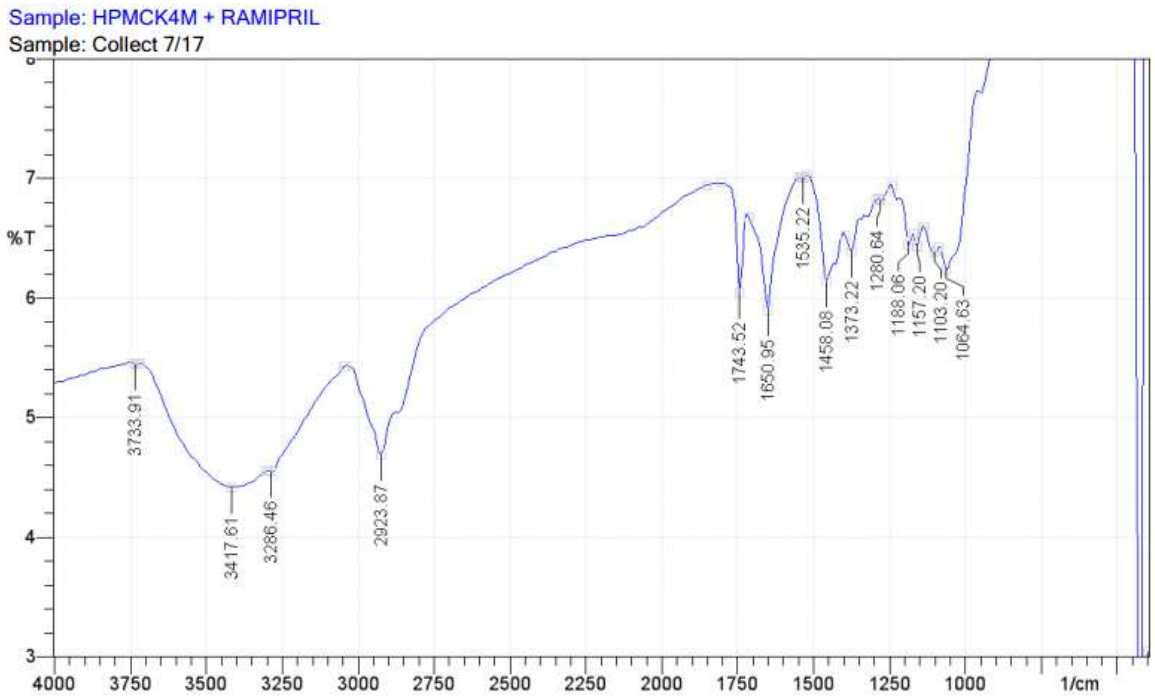


Figure 10. FTIR Spectra of Ramipril and HPMCK4M

Table 15. IR Interpretation of Ramipril and HPMCK4M

Wave Number (cm^{-1})	Interpretation
3733.91	OH stretching
3417.61	NH stretching
2923.87	CH stretching
1650.95	C=OACID stretching
1743.52	C=O ESTER stretching

There is no appearance or disappearance of any characteristic peaks. This shows that there is no chemical interaction between the Ramipril and HPMCK4M.

CALIBRATION CURVE OF RAMIPRIL

The absorbance of the drug in various buffers, 0.1N Hydrochloric acid pH1.2, phosphate buffer pH 7.4 and phosphate buffer pH 6.8 was measured at a wavelength of 210nm. The results are given in table 16.

Table 16. Data for standard curve of Ramipil

S.No	Concentration (mcg/ml)	Absorbance at 210 nm		
		pH 1.2	pH 7.4	pH 6.8
1.	10	0.210	0.455	0.254
2.	20	0.398	0.576	0.476
3.	30	0.610	0.688	0.660
4.	40	0.810	0.803	0.887
5.	50	1.05	0.917	1.140

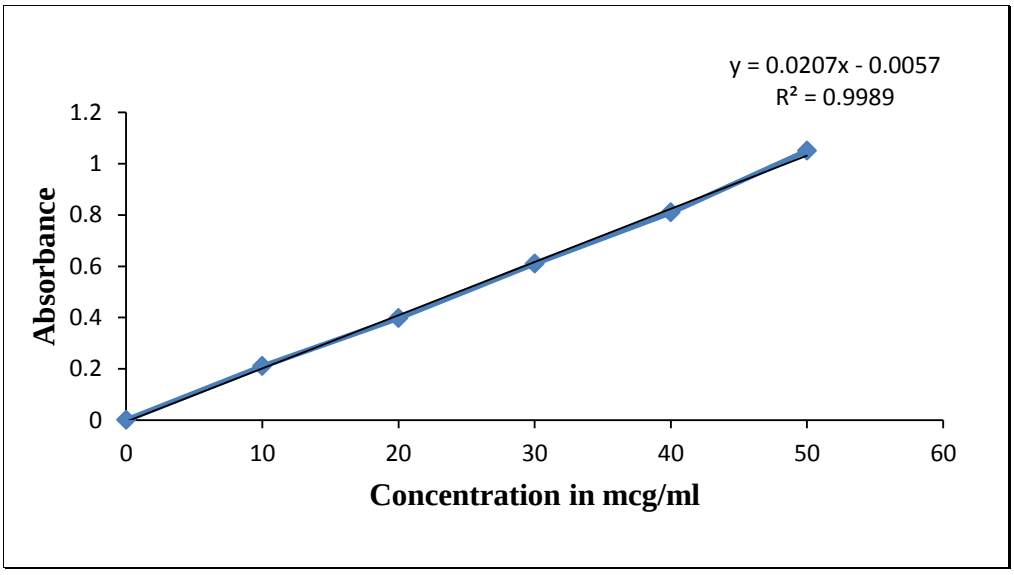


Figure 11. Standard Curve of Ramipril in 0.1 N HCL pH 1.2

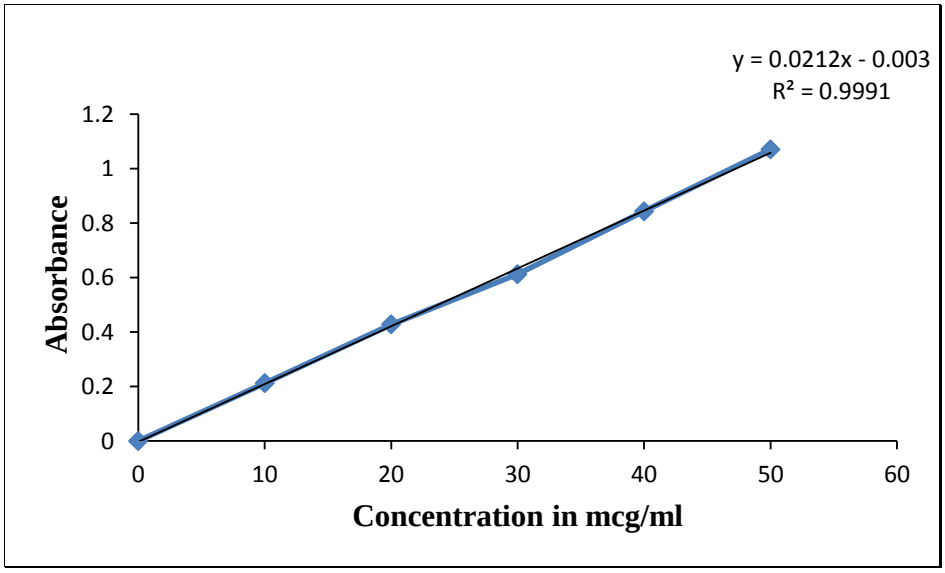


Figure 12. Standard curve of Ramipril in Phosphate Buffer pH 7.4

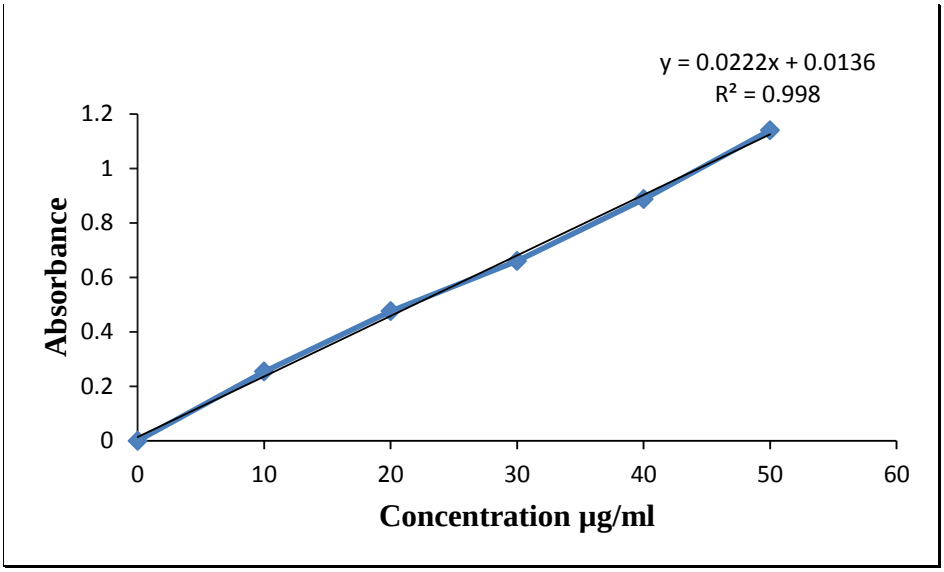


Figure 13. Standard Curve of Ramipril in Phosphate Buffer pH 6.8

The standard curve is linear in the concentration range 0 to 50 mcg/mland starts from the origin. It obeys Beer Lambert'slaw.

PRECOMPRESSION STUDIES OF THE PREPARED MICROSPHERES AND GRANULES

The precompression parameters for the microsphere and granules are given in table 17, 18.

Table 17.Precompression Parameters of Microspheres

Properties	Drug	M1	M2	M3	M4	M5
Angle of repose (θ)	42.15±0.267	33.37±0.2011	33.3±0.7028	33.88±0.4929	34.34±0.3546	33.0±0.3750
Bulk density (g/ml)	1.20±0.0967	0.666±0.00707	0.6±0.005657	0.625±0.007542	0.666±0.007071	0.7±0.009428
Tapped density (g/ml)	1.57±0.0989	0.75±0.08957	0.666±0.007071	0.714±0.009899	0.75±0.00948	0.8±0.01084
Carr's index (%)	23.56±0.9969	11.2±0.988	9.90±0.9076	12.46±0.5666	11.2±0.8485	12.5±0.2854
Hausner's ratio	1.30±0.0998	1.136±0.01053	1.11±0.00282	1.142±0.01517	1.126±0.02248	1.142±0.02248

Mean ±SD(n=3)

The flow property of pure drug was found to be passable and it was identified good flow property for prepared Ramipril microspheres.

Table 18.Precompression Parameters for Granules

Properties	G1	G2	G3	G4
Angle of repose (θ)	34.48±0.0023	33.0±0.0012	34.62±0.0045	33.88±0.0056
Bulk density (g/ml)	0.443±0.0124	0.485±0.0108	0.443±0.01699	0.456±0.01699
Tapped density (g/ml)	0.552±0.0740	0.582±0.01766	0.521±0.0201	0.539±0.0335
Carr's index (%)	18.63±0.008	16.67±0.0235	15.00±0.0162	15.31±0.0202
Hausner's ratio	1.239±0.0128	1.2±0.03536	1.174±0.02166	1.177±0.02577

Mean SD (n=3)

The flow property of granules was found to be good.

Drug content

Drug content of Ramipril granules was analyzed using UV spectrophotometer at 210nm.

Table 19: Drug Content Of Granules

Formulation	% Drug content
G1	94
G2	96
G3	92
G4	98

The drug content of prepared granules was found to be with in pharmacopeial limits.

Scanning Electron Microscopy

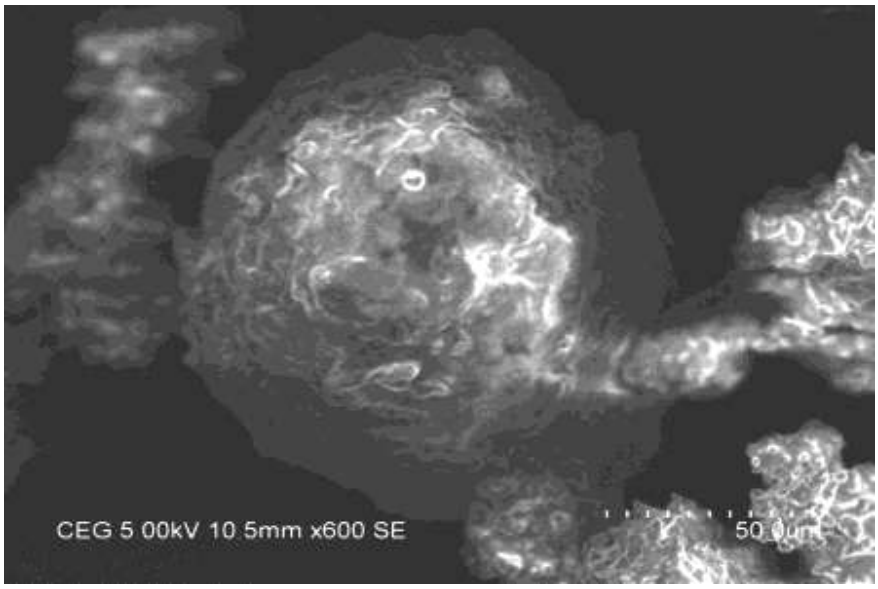


Figure 14. Scanning Electron Microscopy of M4

PERCENTAGE YIELD

Table 20. % Yield of Ramipril Microspheres

Formulation code	Theoretical yield (gm)	Practical yield (gm)	Percentage yield (%)
M1	1	0.87	87
M2	1.5	1.354	90
M3	1.75	1.674	95.65
M4	2	1.983	99.15
M5	2.5	2.451	98.07

After the preparation of microspheres practical yield and percentage yield were calculated. The percentage yield was in the range of 87% to 98.07%w/w.

DRUG CONTENT AND LOADING CAPACITY

Table 21. Drug Content and Loading Capacity

Formulation code	Drug content (%) w/w	Drug Loading (%)
M1	84.4%	11.5%
M2	81.2%	18.6%
M3	83.2%	23.77%
M4	86.3%	35.04%
M5	76.04%	25.09%

The drug content of microspheres ranged from 76.04 to 86.3 % w/w. Formulation M4 contained the maximum drug content.

Evaluation of cross linked empty capsules

Physical Tests

- **Identification**

The '1' size capsules were with purple cap and colourless body. They were lockable type, odorless, softy and sticky when treated with wet fingers. After formaldehyde treatment, there were no significant changes in the capsules. They were non-tacky when touched with wet fingers.

- **Chemical Test**

Qualitative Chemical Test For Free Formaldehyde

The solution was not more intensely coloured then a reference solution prepared at the same time and in the same manner using 1 ml of standard solution. The colour of the test and standard solutions were compared.

PARTICLE SIZE DISTRIBUTION

Table 22.Particle Size Distribution of Formulation M1

Range	Mean Size	No. of particles (n)	n×d	% Frequency
10–20	15	0	0	0
20-30	25	4	100	4
30-40	35	0	0	0
40-50	45	11	495	11
50-60	55	19	1045	19
60-70	65	10	650	10
70-80	75	11	825	11
80-90	85	20	1700	20
90-100	95	25	2375	25
		n=100	nd=7190	Σ=nd/n =71.9µm

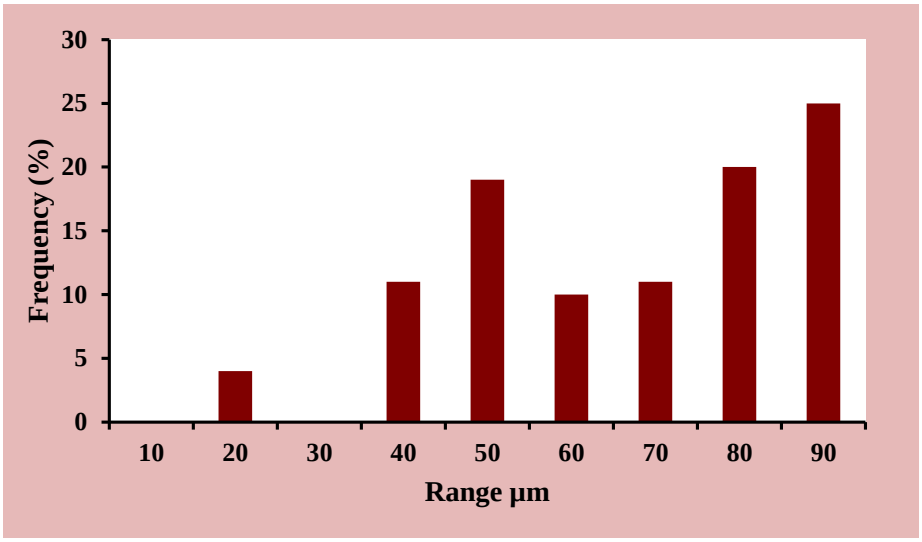


Figure 15. Particle Size Distribution of Formulation M1

The average particle size of M1 was found to be 71.9µm

Table 23.Particle Size Distribution of Formulation M2

Range	Mean Size	No. of particles (n)	n×d	% Frequency
10–20	15	3	45	3
20-30	25	0	0	0
30-40	35	4	140	4
40-50	45	9	405	9
50-60	55	20	1100	20
60-70	65	9	585	9
70-80	75	10	750	10
80-90	85	19	1615	19
90-100	95	26	2470	26
		n=100	nd=7010	Σ=n/nd=70.10µm

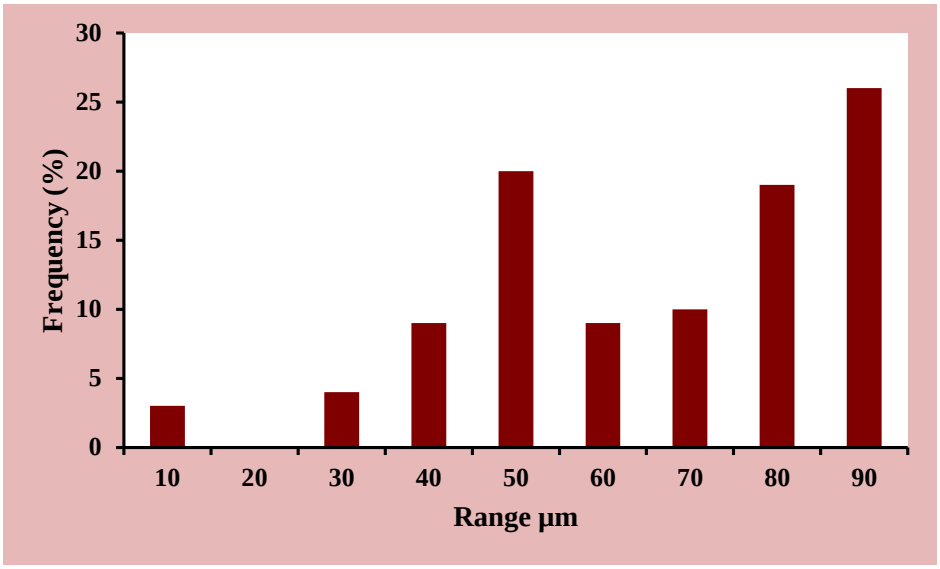


Figure 16. Particle Size Distribution of Formulation M2

The average particle size of M2 was found to be 70.10µm.

Range	Mean size	No.of particles (n)	n×d	% Frequency
10–20	15	2	30	2
20-30	25	0	0	0
30-40	35	3	105	3
40-50	45	9	405	9
50-60	55	19	1045	19
60-70	65	11	715	11
70-80	75	10	750	10
80-90	85	20	1700	20
90-100	95	26	2470	26

Table 24. Particle Size Distribution of Formulation M3

n=100 nd=7220 Σ=n/nd=72.2µm

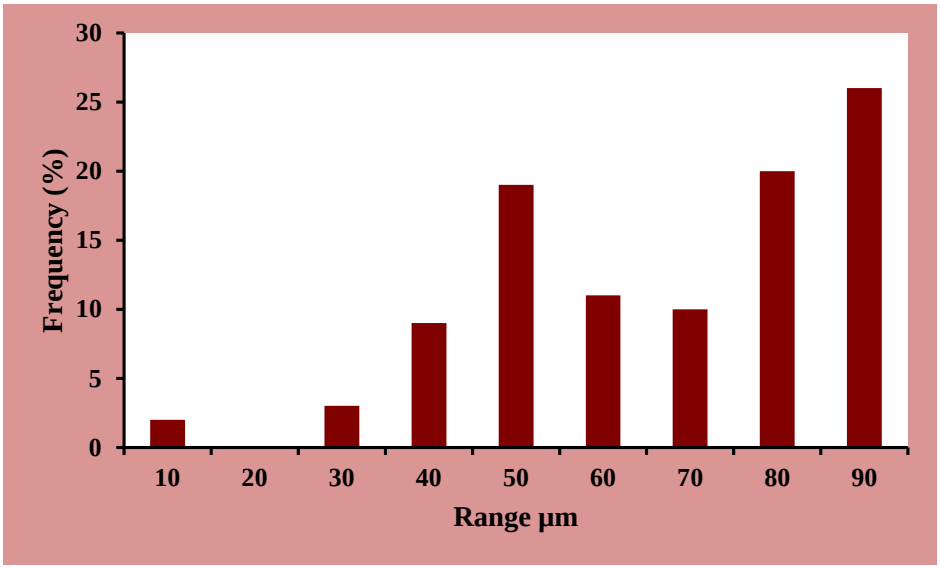


Figure 17. Particle Size Distribution of Formulation M3

The average particle of M3 was found to be 72.20µm

Table 25. Particle Size Distribution of Formulation M4

Range	Mean Size	No. of particles (n)	n×d	% Frequency
10–20	15	0	0	0
20-30	25	4	100	4
30-40	35	2	70	2
40-50	45	2	90	2
50-60	55	25	1375	25
60-70	65	20	1300	20
70-80	75	10	750	10
80-90	85	17	1445	17
90-100	95	20	1900	20
		n=100	nd=7030	Σ=n/nd=70.30µm

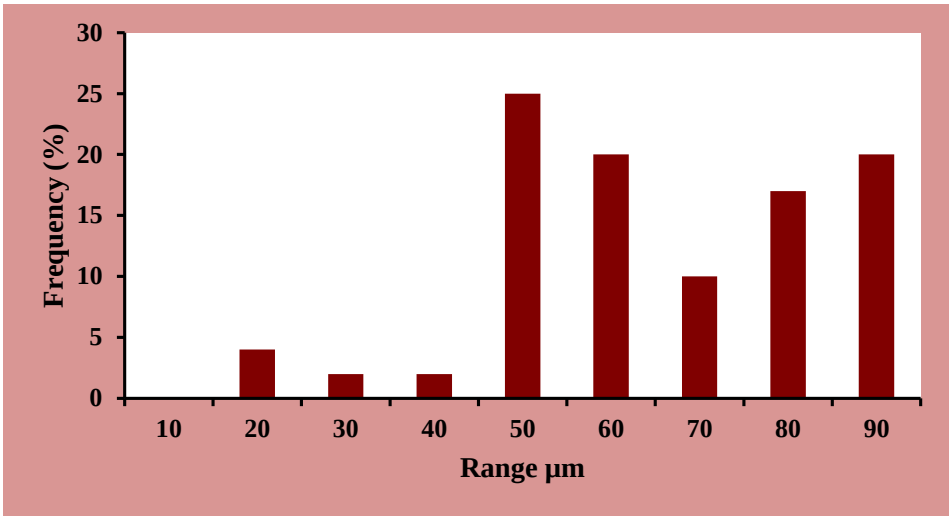


Figure 17. Particle Size Distribution of Formulation M4

The average particle size of M3 was found to be 70.30µm.

Table 26. Particle Size Distribution of Formulation M5

Range	Mean Size	No. of particles (n)	n×d	% Frequency
10–20	15	2	30	2
20-30	25	0	0	0
30-40	35	6	210	6
40-50	45	8	360	8
50-60	55	19	1045	19
60-70	65	10	650	10
70-80	75	12	900	12
80-90	85	21	1785	21
90-100	95	22	2090	22

n=100 nd=7070 Σ=n/nd=70.70µm

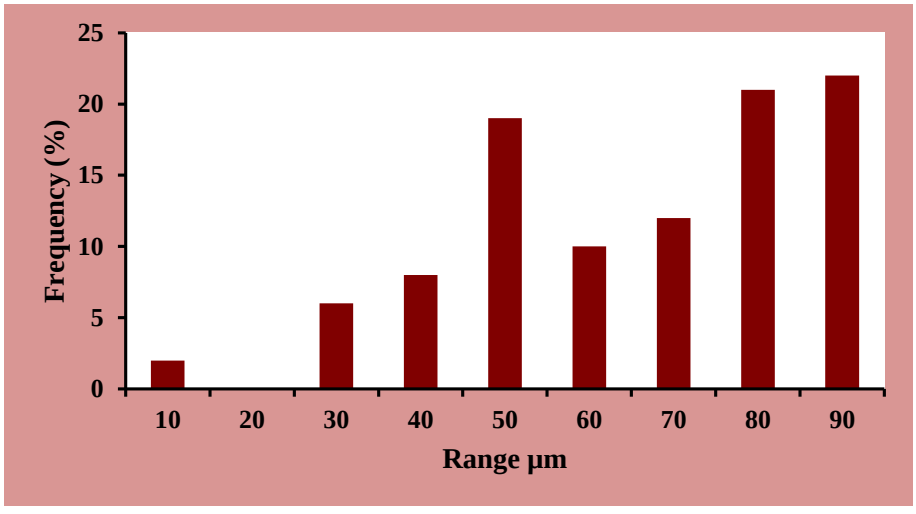


Figure 19. Particle Size Distribution of Formulation M5

The average particle size of M5 was found to be 70.70µm.

EVALUATION PARAMETERS FOR HYDROGEL PLUG

Table.27 Evaluation Of Hydrogel Plug

Hydrogel plug code	Weight (mg)	Thickness (mm)	Hardness
P1	100mg	3.20 ±0.0023	2.4 ±0.0056

Table. 28 Swelling index

pH	Time(Hrs)	% Swelling index
pH 1.2	2	33.11
	4	52.38
	6	64.82
	8	70.29
	10	72.22
	12	75.48
pH 7.4	2	37.65
	4	54.09
	6	65.03
	8	70.88
	10	72.58
	12	75.77
pH 6.8	2	37.26
	4	53.66
	6	64.70
	8	70.97
	10	73.04
	12	76.07

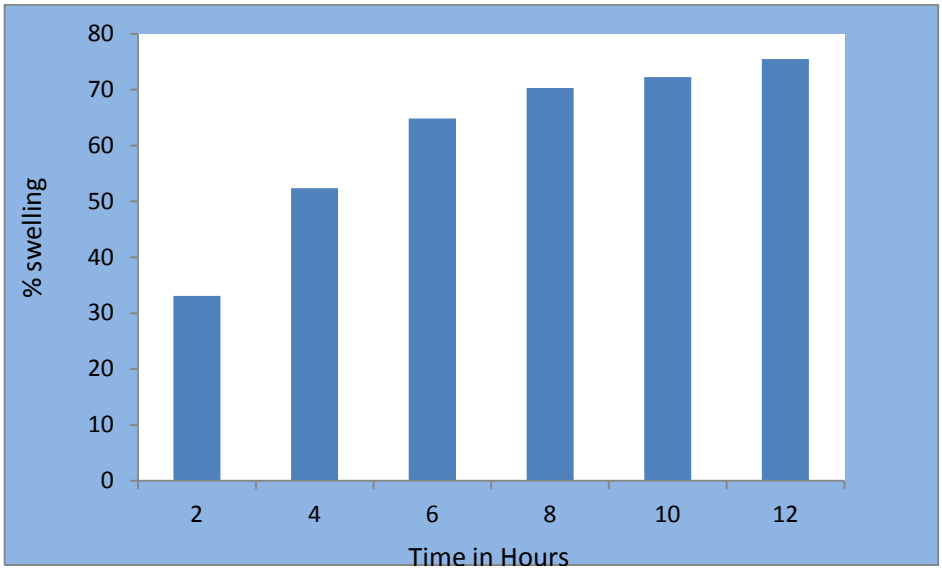


Figure 20. Swelling index in 0.1 N HCL

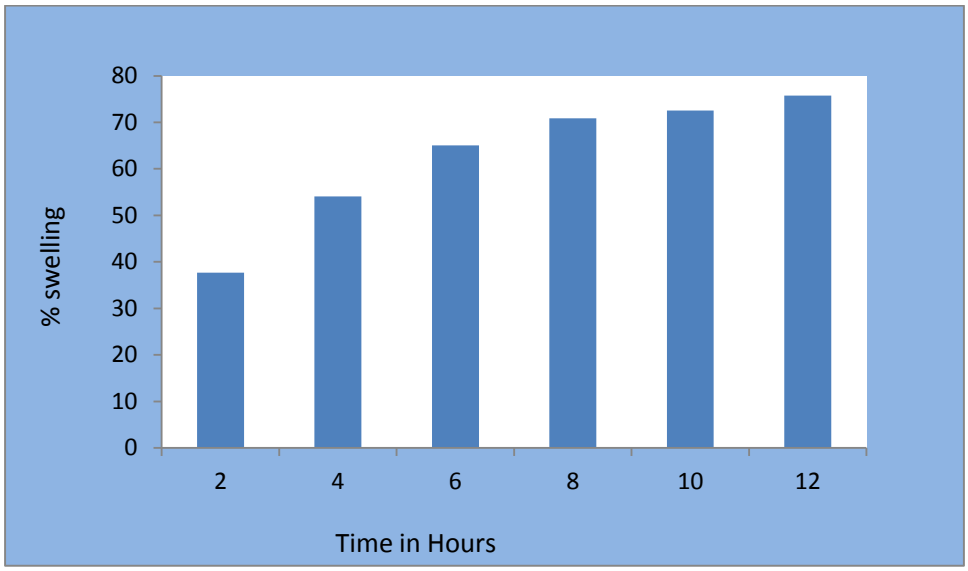


Figure 21. Swelling index in phosphate buffer pH 7.4

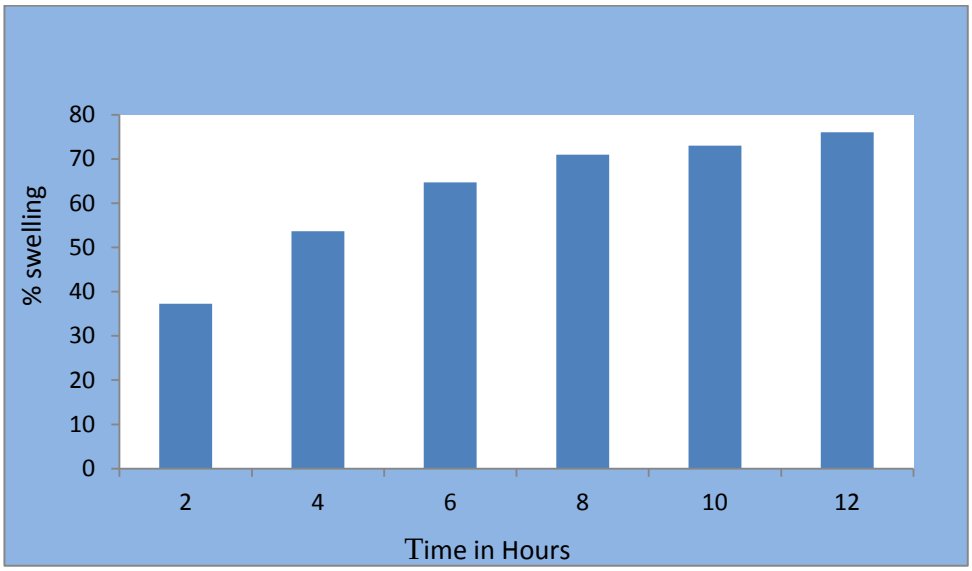


Figure 22. Swelling index in phosphate buffer pH 6.8

Swelling index of HPMCK4M hydrogel plug showed the plug integrity and increased swelling during the study.

TABLE 29.*IN VITRO* DISSOLUTION OF RAMIPRIL IR

Time in minutes	Cumulative % drug release			
	G-1	G-2	G-3	G-4
10	30.6±0.236	34.88±0.442	38.87±0.447	38.52±0.132
20	36.68±0.583	73.15±0.246	79.18±0.242	72.84±0.612
30	61.6±0.134	87.64±0.463	94.56±0.293	**101.59±0.413**
40	75.88±.123	92.2±0.674	**101.67±0.473**	
50	90.07±.314	**102.68±0.349**		
60	**101.78±0.213**			

Mean SD (n=3)

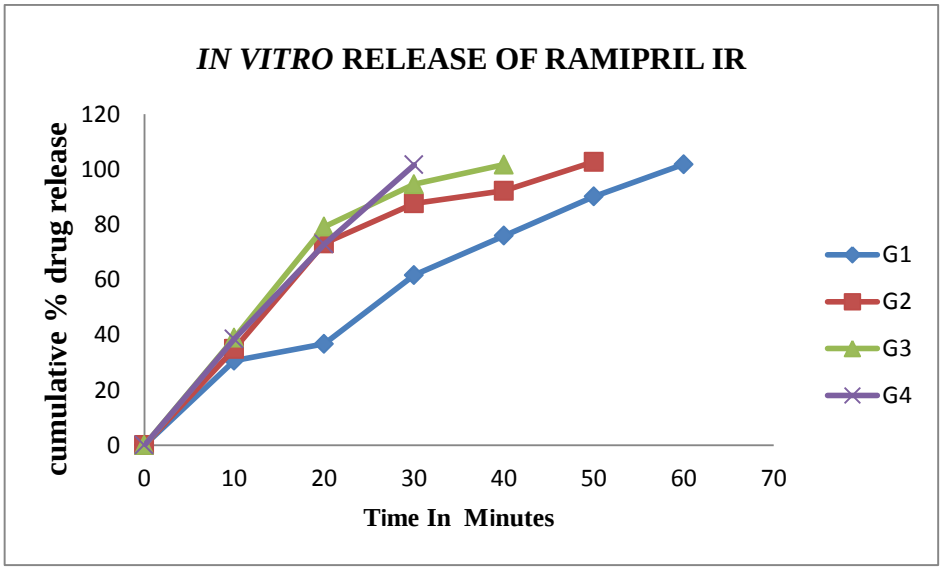


Figure 23.*In vitro* dissolution of Ramipril IR

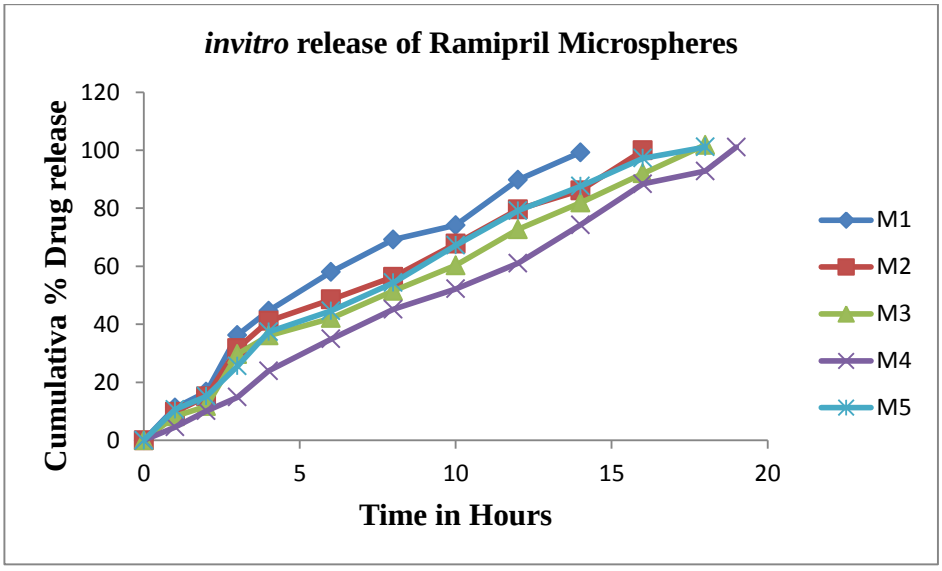
The in vitro dissolution of Ramipril IR showed that G4 was found to be optimum for immediate release.

TABLE 30. *IN VITRO* DISSOLUTION OF RAMIPRIL MICROSPHERES

Time in Hours	Cumulative % drug release				
	M-1	**M-2**	**M-3**	**M-4**	**M-5**
1	11.2±0.098	9.76±0.586	8.26±0.098	4.56±0.067	10.62±0.023
2	16.68±0.167	15.08±0.235	11.72±0.076	10.11±0.0789	15.2±0.197
3	36.28±0.056	31.72±0.067	29.69±0.309	14.86±0.543	25.59±0.942
4	44.6±0.087	41.12±0.054	36.12±0.209	23.91±0.0721	37.39±0.621
6	58.02±0.065	48.52±0.452	42.12±0.120	34.9±0.129	44.62±0.185
8	69.2±0.128	56.32±0.234	51.47±0.521	45.16±0.284	54.32±0.049
10	74.12±0.112	67.72±0.601	60.27±0.045	52.16±0.492	67.21±0.183
12	89.76±0.078	79.6±0.067	72.71±0.098	61.01±0.719	79.14±0.061
14	**99.21±0.478**	86.12±0.087	81.92±0.067	74.21±0.497	87.56±0.674
16		**99.92±0.012**	91.99±0.390	88.41±0.045	97.26±0.184
18			**101.77±0.865**	92.76±0.729	**101.08±0.295**
19				**101.02±0.571**	

Mean SD (n=3)

Figure 24.*In vitro* dissolution of Ramipril IR



The invitro release of Ramipril microspheres was evaluated. M4 have more sustained release than all the formulations therefore M4 was optimized.

TABLE 31.*IN VITRO* RELEASE OF RAMIPRIL PULSINCAP

Dissolution Medium	Time	% Cumulative Drug Release
0.1N HCL pH 1.2 Buffer	10	18.84±0.169
	20	36.47±0.278
	30	50.67±0.061
	40	51.26±0.037
	50	51.82±0.028
	60	51.51±0.069
	90	53.03±0.119
	120	53.67±0.043
pH 7.4 Buffer	3	0
	4	0
	5	0
pH 6.8 Buffer	6	4.95±0.042
	7	6.74±0.016
	19	32.50±0.075
	20	38.61±0.113
	21	41.45±0.0478
	22	45.46±0.171
	23	48.41±0.004
	24	**50.30±0.063**

Mean SD (n=3)

Figure 25.*In vitro* release of Ramipril Pulsincap

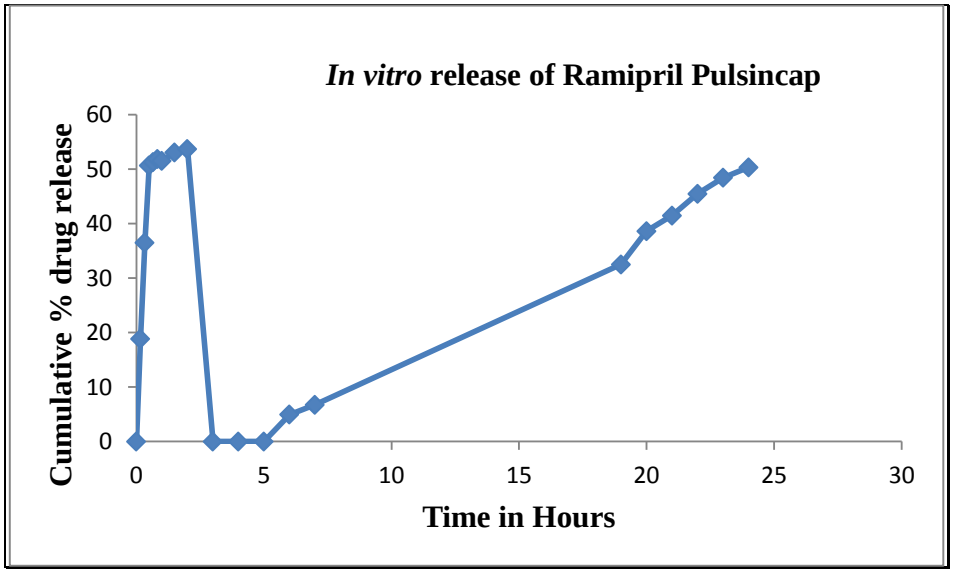


Table 30. Release Kinetics of Ramipril Pulsincap

Time (Hrs)	Log Time (Hrs)	Sq.root of time (Hrs)	Cum % drug release	Cum% drug remaining	Log Cum % drug release	Log cum % drug remaining	Cube root of cum % drug remaining
0	-∞	0	0	50	-∞	1.698	3.68
6	0.778	2.449	4.98	45.02	0.697	1.653	3.557
7	0.845	2.645	6.76	43.24	0.829	1.635	3.509
19	1.278	4.358	32.06	17.44	1.512	1.241	2.593
20	1.301	4.472	38.77	11.23	1.588	1.050	2.239
21	1.322	4.582	41.43	8.57	1.614	0.932	2.046
22	1.342	4.690	45.26	4.74	1.655	0.675	1.679
23	1.361	4.795	48.14	1.86	1.682	0.269	1.229
24	1.380	4.898	50.24	0.24	1.701	0.619	0.621

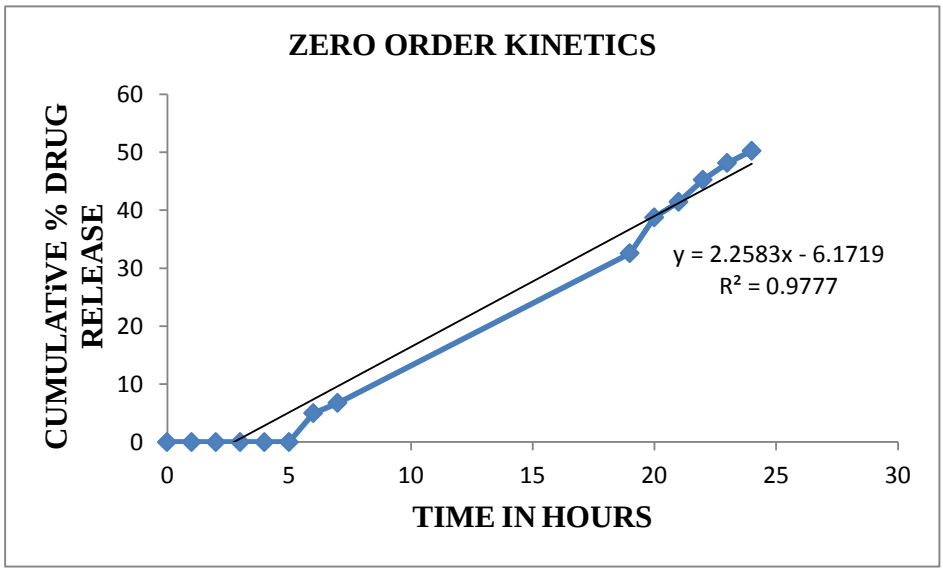


Figure 26 : A Plot Of Zero Order Kinetics

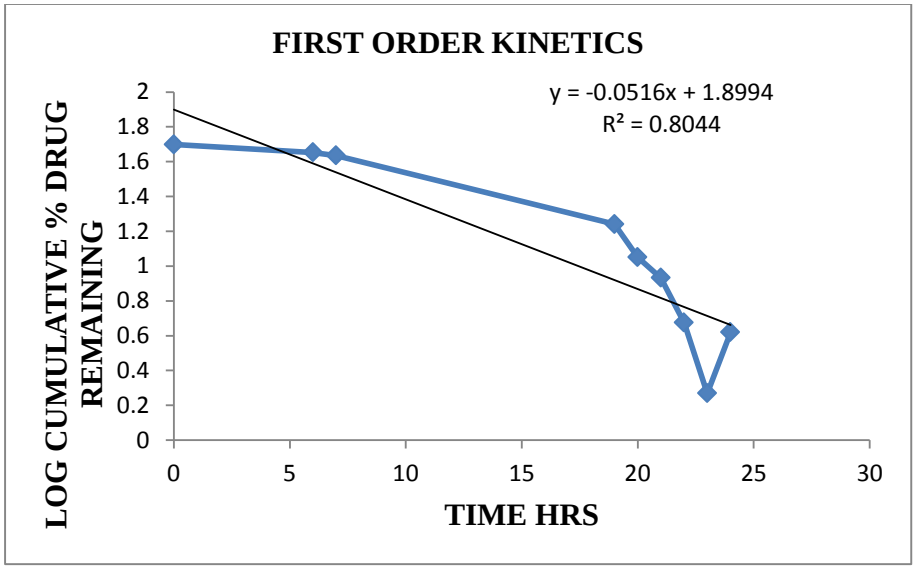


Figure 27 : A Plot First Order Kinetics

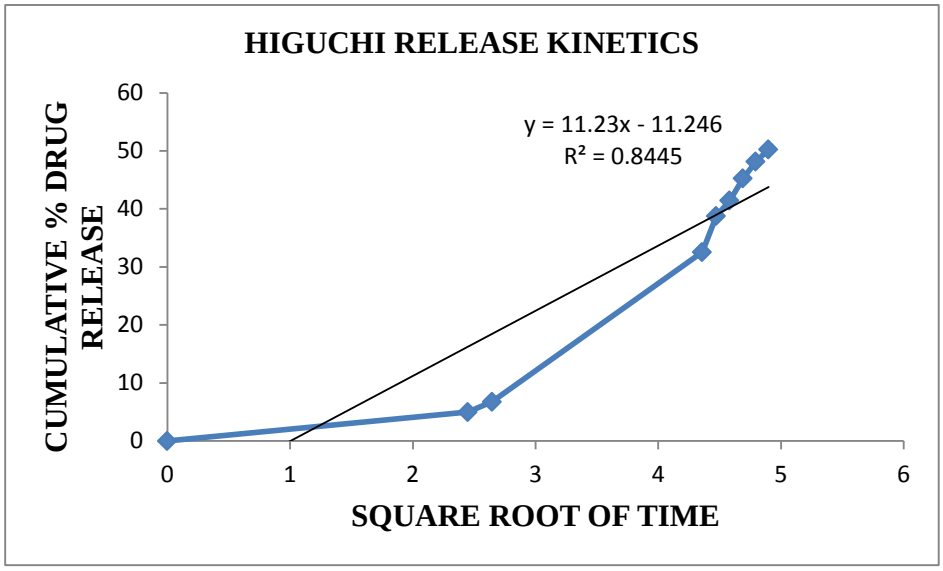


Figure 28: A Plot Higuchi Release Kinetics

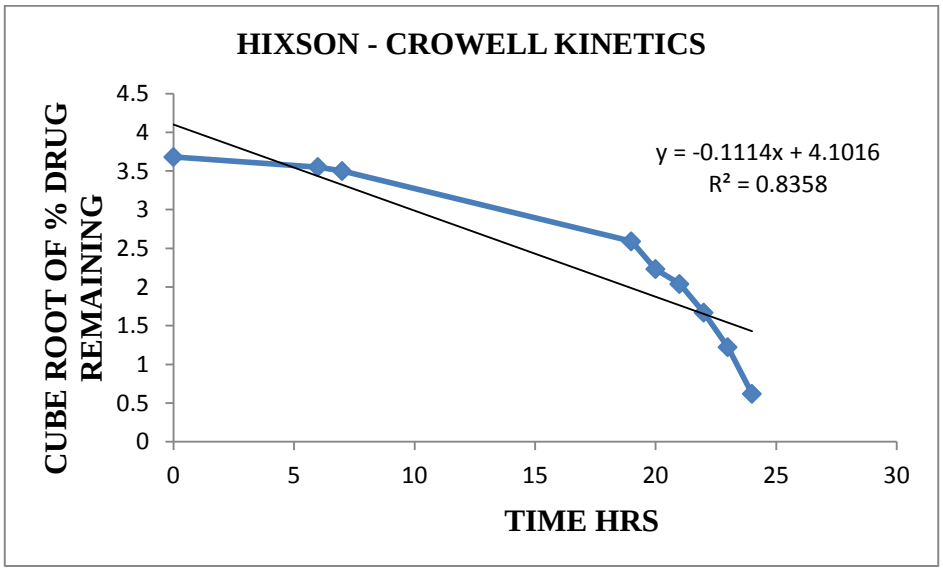


Figure 29: A PlotHixson- Crowell Kinetics

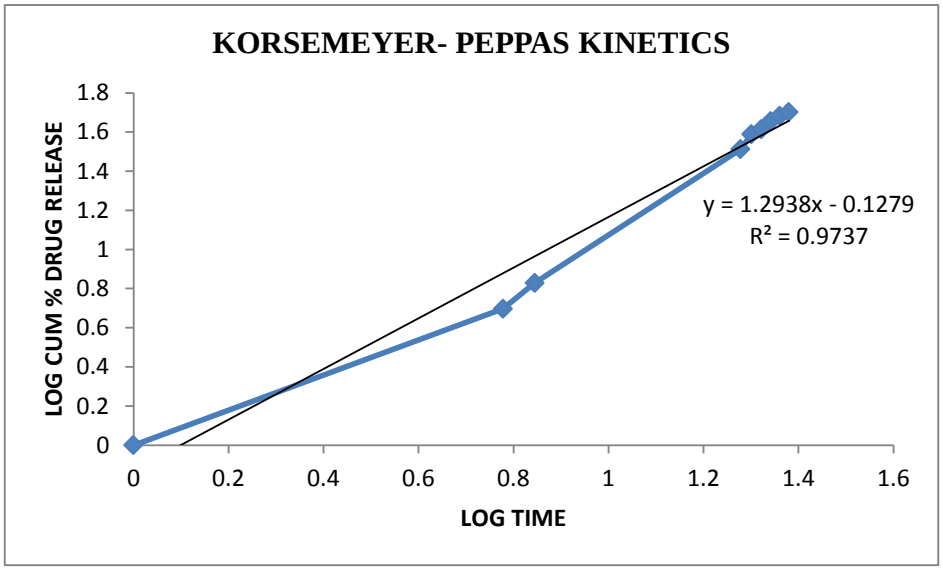


Figure 30: A Plot Korsemeyer-Peppas Kinetics

Determination of drug release mechanism of optimized Ramipril Pulsincap

- The order of release was found to be zero order in which R^2 value was close to 1 . So the formulation follows zero order kinetics followed by Korsemeyer-peppas model.
- The mechanism of drug release was found to be diffusion and dissolution.

Stability study:

The optimized pulsincap were subjected to stability studies and the results are given in Table 31.

Table 31: Drug content and Dissolution profile of Ramipril

Time interval (month)	Drug content (%)		Cumulative % release	
	Immediate release Granules	Microspheres	Immediate release Granules	Microspheres
1 month	98.70±0.016	86.53±0.021	53.45±0.044	51.70±0.148
2 month	98.99±0.022	86.73±0.036	52.99±0.054	51.31±0.048
3 month	97.23±0.033	85.01±0.024	53.53±0.014	51.20±0.014

Mean ±SD (n=3)

No significant changes were observed in the physical appearance, colour, drug content and drug release of Ramipril pulsincap of the optimized batch at 40^0C /75% RH. The Ramipril pulsincap are stable.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Ramipril is an antihypertensive drug belongs to the class of angiotensin converting enzyme inhibitor (ACE).It has relatively poor pharmacokinetic profile with 28-35% absolute bioavailability undergoing extensive first pass metabolism. It has half life of 2-4hrs. To overcome these drawbacks and to release the drug in a sustained manner, Ramipril was formulated as microspheres by Emulsion polymerization technique using Egg Albumin as polymer .The immediate release was prepared as granules by wet granulation method.

- Preformulation studies were performed for microspheres and granules. The flow properties were found to be good.
- Physical compatibility study showed that the drug and excipient were physically compatible with each other.
- Chemical compatibility study using FTIR spectroscopy revealed no interaction between the drug and excipients.
- The capsule body was made insoluble by formaldehyde treatment and were subjected to various physical and chemical test such as dimension measurement, solubility studies and qualitative for free formaldehyde.
- Hydrogel plug was prepared with 9mm punch and post compression study was performed.
- The size of microspheres was confirmed by SEM.
- The *in vitro* release studies were preformed for all the formulations. G – 4 showed 101.59 % at 30 minutes , M – 4 showed 101.02% at the end of 19^{th} hour. Therefore it was chosen as the optimized formulation for pulsincap.
- The *invitro* release of pulsincap for optimized formulation were performed.
- The accelerated stability testing was carried out for 3 months and was found to be stable.

FUTURE SCOPE

- *In -vivo* study using gamma scintillography method
- Pharmacokinetic and toxicity study
- Stability studies-long term

REFERENCES

REFERENCE

1. Chein YW. Novel drug delivery systems. New York. Marcel dekker. Inc., 2nd edition; 1992;50:139-140.
2. Deepika jain , Raicha Raturi, vikas Jain, Praveen Bansal , Ranjit Singh. Recent technologies in pulsatile drug delivery. *Biomatter* 2011;1:1, 1-9; July/August/September.
3. Raghavendra rao N G, Soumya P , Revathi , Sanjeev Nayak B . A review on pulsatile drug delivery system. *International Research Journal of Pharmacy.* 2013; 4 (3).
4. Rawat S, Bisht S , Kothiyal P. Pulsatile drug delivery " A Programmed Polymeric device ". *Indian Drugs* . May 2013;50(05).
5. Supriya. Shidhaye, Ashwini. Dhone, Tejal. Budhkar ,Chaitali. Surve. Technologies in Pulsatile Drug Delivery System. *International Journal Of Advances In Pharmacy, Biology And Chemistry*. Oct- Dec 2012: Vol. 1(4).
6. Tajane SR , kholwal BB , suryawanshi SS , Tarkase KN . Current Trends In Pulsatile Drug Delivery Systems. *International Journal Of Pharmaceutical Sciences And Research.* 2012; Vol. 3(2): 358-366.
7. Jigar D. Patel, Kritika Aneja, Shivprasad H. Majumdar. Pulsatile Drug Delivery System: An "User-Friendly" Dosage. *JPRHC* .April 2010; Volume 2 (2) : 204-215 .
8. Anantha Nayaki Ravula*, Bairi Agaiah Goud A .Review On - Recent Advances In Oral Pulsatile Drug Delivery. *Journal Of Advanced Pharmaceutical Sciences* ; 2011 Vol-1 (1).
9. Alagusundaram M , Madhu Sudana Chetty C, Umashankari K, et al., Microspheres as a Novel Drug Delivery System- A Review. *International .Journal of ChemTech Research.* 2009;1(3):526-534.
10. Sarlesh rajput , Preeti agarwal, Ashish Pathak, Nikhil Shrivasatava, Satyendra Singh Baghel, Rajendra singh baghel . A Review On Microspheres: Methods Of Preparation And Evaluation .*World journal of pharmacy and pharmaceutical sciences.* June 2012; Volume 1 Issue 1: 422-438.

REFERENCE

11. Kataria Sahil, Middha Akanksha, Sandhu Premjeet, Ajay Bilandi , Bhawana Kapoor. Microsphere: A Review. *International Journal Of Research In Pharmacy And Chemistry*. 2011 ;1(4).
12. Felix Kratz . Albumin As A Drug Carrier : Design Of Prodrugs, Drug Conjugates And Nanoparticles. *Journal Of Controlled Release.* 2008: 171 - 183
13. Ashvini veeraraje urs , Kunchu Kavitha, Ganesh Nanjan Sockan. Albumin Microspheres; An Unique System As Drug Delivery Carriers For Non Steroidal Ant-Inflammatory Drugs. *International Journal Of Pharmaceutical Sciences Review And Research.*November – December 2010; vol 5 (2):10-17.
14. Listair CR, Rossjm, Mathias W, Howardnes. Chronopharmaceutical Drug Delivery From A Pulsatile Capsule Device Based On Programmable Erosion. *J pharm.pharmacol* 2002;52:903-909.
15. Anil Kumar. A, Rajyalakshmi. Formulation And Evaluation Of Metoprolol Succinate Pulsatile Drug Delivery System For Chronobiology Disorder: Anti Hypertensive. *International Journal Of Pharmaceutical Sciences And Research.* 2012; Vol.3(10):4004-4009.
16. Vihar moturi et al. Formulation and in Vitro Evaluation of Modified Pulsincap of a Cardiovascular Drug. *Asian journal of pharmacy and life science*. Jan-march, 2013; 3(1.)
17. Hakan Erogul, Suheyla Kas, Levent Oner, Mustafa Sargon, Atilla Hincal A. Preparation Of Bovine Serum Albumin Microspheres Containing Dexamethasone Sodium Phosphate And The In Vitro Evaluation. *Turk J Med Sci*. 2000; 30 : 125-128.
18. Meena A, Kumar B, Suriya Prakash T N K, Senthamarai R. Development And Evaluation Of Pulsatile Drug Delivery System Of Lornoxicam. *International Journal Of Pharma World Research.* Mar-Jun 2011; Vol2(2).
19. Sukanya M, Sai Kishore V . Design And Development Of Chronopharmaceutical Drug Delivery Of Simvastatin. *Journal Of Chemical And Pharmaceutical Research*.2012;4(6):3195-3200.

REFERENCE

20. Senthilnathan B, Aviyankavasireddy , Kausalya J, Suba.V. Design And Development Of Pulsatile Drug Delivery Of Glibenclamide Using Pulsincap Technology. *International Journal Of Pharmtech Research.* Oct – Dec 2012; Vol 4(4):1606-1613.
21. Najmuddin.M , Vishal Ashok Patel, Azgar Ali, Shelar S, Tousif K. Development And Evaluation Of Pulsatile Drug Delivery System Of Flurbiprofen. *Research Journal Of Pharmaceutical Biological And Chemical Sciences*. April-June 2010; Vol 1 (2):284-290.
22. Amol M . Design And Evaluation Of Pulsatile Drug Delivery System Of Atenolol For Chronomodulated Therapy. *International Journal Of Pharma And Bio Sciences*. 2012 Oct; 3(4):1-8.
23. Sindhu Abraham , Srinath MS. Development of modified pulsincap drug delivery system of diclofenac for drug targeting . *Indian Journal Of Pharmaceutical Science.* 2007;69(1):24-27.
24. Masa Reddy, S Susmitha, A Gupta, A D'Souza, " chronomodulated pulsincap drug delivery system of aceclofenac ", Inventi Rapid: NDDS , Vol. 2011.
25. Howard NE Stevens et al**.**Evaluated the pulsincap to provide regional delivery to dofetilide to the human GI tract. *International Journal Of Pharmaceutics*.2002;1(2);27-34.
26. Neha Manish Munot , Preeti Prasad Lasure, Grime S S. Design And Evaluation Of Chronotropic System For Colon Targeted Drug Delivery. *International Journal Of Pharmacy Research And Technology.* 2012;Vol2 (3):13-17.
27. Muniswamy P , Jagannath M, Harisa M , Shanta Kumar S M , Putta Rajesh Kumar. Verapamil Hydrochloride Systems Design For Pulsincap Drug Delivery : Development And Evaluation Studies. *Journal of biomedical and pharmaceutical research.* 2012;1(3):101-109.
28. Srinivas L et al**.** studied Ibuprofen as pulsincap technique. *International Journal Of Pharmacy Research And Technology.* 2011;Vol1(3):12-17.
29. Dinal Patel, Nirav Patel, Vaishali Thakkar, Ashok Modi, Tejal Gandhi. Development And Characterication Of Mucoadhesive Microspheres Of Levlsalbutamol Sulphate. *International Journal Of Pharmaceutical Sciences And Research.* 2013;vol 4 (5):1838-1851.

REFERENCE

30. Arora Neha , Khattar Himanshi, Parashar Deepa, Arora Nageen, Garg Tarun. Evaluation Of Entrapment Efficiency Of Glipizide Microsphere. *Iosr Journal Of Pharmacy.* Mar-Apr 2012; Vol2(2):180-181.
31. Senthil A , Sivakumar T. Design And Characterization Of Mucoadhesive Glipizide Microspheres. *Pharmacologyonline.* 2011;1:85-98.
32. Mishra Manoj Kumar, Kamboj Vipan , Verma Prabhakar , Nigam Vijay. Preparation And Evaluation Of Biodegradable Albumin Microspheres Of Ketorolac Tromethamine. *International Research Journal Of Pharmacy.*2011; 2(1):214-218.
33. Vikas Parashar , Dabeer Ahmad, Surya Prakash Gupta, Neeraj Upmanyu, Neha Parashar, Vinod Mudgal. Formulation And Evaluation Of Biodegradable Microspheres Of Tinidazole. *International Journal Of Drug Delivery System.* 2010;2:238-241.
34. Ashvini Urs V , Kavitha K , Mehaboob Y. Design And Evaluation Of Ketoprofen Loaded Albumin Microspheres. *An International Journal Of Pharmaceutical Sciences.* July 2011; Vol 2 (3):189-203.
35. Aydan Gulsu , Hakan Ayhan , Fatma Ayhan . Preparation And Characterization Of Ketoprofen Loaded Albumin Microspheres. *Turkish Journal Of Biochemistry.* 2012;37(2):120-128.
36. Chinna Gangadhar B , Shyam Sundar R, Vimal Kumar Varma M, Sleeve Raju M, Sai Kiran M. Formulation And Evaluation Of Indomethacin Microspheres Using Natural And Synthetic Polymers As Controlled Release Dosage Forms. *Interanational Journal Of Drug Discovery.* 2010; Vol 2 (1):08-16.
37. Sayyed Abolghassem Sajadi Tabassi, Naheed Razavi. Preparation And Characterization Of Albumin Microspheres Encapsulated With Propranolol Hcl . *Daru*. 2003; Vol 11(4).
38. Yaminipendyala, Sudha Talasila . Formulation And Evaluation Of Chitosan Loaded Mucoadhesive Microspheres Of Ramipril. *International Journal Of Pharmaceutical And Chemical Science.* Jul – Sep 2012; Vol1(3):555-562
39. Dandagi Pm, Mastiholimath V S , Patil M B , Gupta M K . Biodegradable Microparticulate System Of Captopril. *Pubmed.* Jan 2006;307(1).

REFERENCE

40. Ravi S Wanare . Enhancement Of Dissolution Rate Of Poorly Water Soluble Drug By Solid Dispersion Technique. *Research Journal Of Pharmaceutical, Biology And Chemical Sciences.* April-June 2013; Vol 4 (2):686-707.
41. Sudheer Nadendla, Snehalatha, Nagaraja T S , Bharathi. Effect Of Various Polymers On Swelling And In Vitro Release Of Ramipril In Effervescent System. *International Journal Of Pharmacy And Life Sciences.* 2013 April;4(4).
42. Harish Chander, Sachin Kumar , Bineeta Bhatt. Formulation And Evaluation Of Fast Dissolving Tablet Of Ramipril. *Pelagia Research Library*. 2011;2(6):153-160.
43. Sivannarayana P, Prameela Rani A, Saikishore V. Formulation And Evaluation Of Ramipril Sr Matrix Tablets By Using Tamirndus Kernel Mucilage As Release Retardant. *Research Journal Of Pharmaceutical, Biological And Chemical Sciences.* July - September 2013;Vol 4(3):1432-1440.
44. Rajesh A , Balasubramaniam V , Velrajan G, Srinivas D , Vidayasar K E C. Formulation And Evaluation Of Mouth Dissolving Tablets Of Ramipril. *Asian Journal Of Pharmaceutical Research.* 2013;Vol 3(1):9-14.
45. Sachin Patil, Shilpa Chaudhari, Ratnaparkhi M P . Formulational And Evaluation Of Beta Blocker And Ace Inhibitor Bilayered Tablet By Direct Compression Technique. *Der Pharmacia Letter*. 2013; 5(4):164-172.
46. Ratnaparkhi Mukesh, Dhage Kiran, Chaudhari Shilpa, Salvankar Shellaendra. Formulation And Evaluation Of Immediate Release Tablets Of Metformin Hcl And Glibenclamide Using Different Superdisintegrants. *Phtechmed.* May – June 2012; Vol1(3):81-86.
47. Koda-Kimble, Mary Anne; Young, Lloyd Yee; Alldredge, Brian K.; Corelli, Robin L.; Guglielmo, B. Joseph; Kradjan, Wayne A.; Williams, Bradley R. *Applied Therapeutics: The Clinical Use Of Drugs.* 9th Edition. P.13p2-P.13p10
48. mhtml:file:// I:\Drug Bank Ramipril (DB00178).mht.
49. Rowe, R.C. Etal. *Handbook Of Pharmaceutical Exicipents*. London: Pharmaceutical Press And American Pharmacists Association . 2009.
50. Banker GS, Anderson NR. (1987). Tablets in :Lachman L, Lieberman HA, Kanig JL, ed. The theory and practice of industrial pharmacy. 3rd ed. Mumbai : Varghese publishing house, 182-4;296-303:311-2.

REFERENCE

51. Leo lachman, Herbert A Liberman , Joseph L Kanig. The theory and practice of industrial pharmacy 3rd Edition. Lea and Feibger 1990; 296-299.
52. Ministry of health and family welfare. Indian pharmacopoeia. Ghaziabad: the indianpharamacopoeial commission; 1996. Vol.11.p.182.
53. Kannan S et al. Formulation And Evaluation Of Aspirin Delayed Release Tablet. Phatmacie Globale : International Journal Of Comprehensive Pharmacy. 2010;1(4):13.